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INACTIVATION OF HEPATITIS A VIRUS (HAV) BY CHLORINE AND IODINE IN WATER

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The inactivation kinetics of hepatitis A virus (HAV), poliovirus 1 (P1) and echovirus 1 (E1) by free chlorine and Army iodine (globaline) were studied in phosphate buffered, halogen demand free-water (PBDFW) and in PBDFW supplemented with 5 NTU of clay tubdidity and 10 mg/l humic+fulvic acids. HAV was rapidly inactivated by free chlorine under all conditions tested (1-7 mg/L, 5 and 25°C, pH 4.5, 7.0 and 9.5), with 99.99% reductions in <8 minutes. P1 and E1 were inactivated more slowly than HAV but by >99.99% under most conditions tested. Iodine at 1 and 2 tablets per quart was effective at pH 9.5, with >99.99% inactivation of all three viruses in <10 minutes. However, at pH 4.5 and 7.0 and 5°C, iodine inactivation of P1 and E1 was sometimes slow. Iodine may not inactivate HAV and other enteroviruses effectively under some field conditions.

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SUMMARY

The inactivation kinetics of hepatitis A virus (strain HM175), poliovirus 1 (strain LSc) and echovirus 1 (strain V239) by free chlorine and Army iodine (globaline) were determined in batch laboratory experiments. Virus preparations contained a defined distribution of various sized aggregated particles. Disinfectant doses and test waters were 1 and 5 mg/l free chlorine and 1 and 2 tablets per quart (about 8 and 16 mg/l) of globaline in phosphate buffered halogen demand-free water (PBDFW) and 3 and 7 mg/l free chlorine and 1 and 2 tablets per quart of globaline in "worst case" water. Test waters were studied at pH 4.5, 7.0 and 9.5 and 5 and 25°C. Worst case water was PBDFW containing 5 NTU of bentonite clay turbidity and 10 mg/l of a 1:1 mixture of aquatic humic and fulvic acids. The inactivation kinetics of three strains of HAV (HM175, MD-1 and CR326) by free chlorine and globaline were compared at selected conditions of pH (4.5 and 9.5) and temperature (5 and 25°C).

HAV was inactivated relatively rapidly by free chlorine under all conditions tested, with times for 99.99% inactivation (T-99.99) of <8 minutes. Polio 1 and echo 1 were also inactivated rapidly by free chlorine at pH 4.5 and 7.0, with T-99.99 values of 7.0 minutes or less. However, polio 1 and echo 1 were inactivated slowly by 1 mg/l free chlorine at pH 9.5 and 5°C (T-99.99 values of 57 minutes or more). Virus inactivation by free chlorine was generally more effective at lower pH, higher temperature and higher chlorine dose. Virus inactivation kinetics differed in PBDFW and worst case water, but this effect was probably related to differences in chlorine doses. The three strains of HAV tested were quite similar in response to free chlorine. If free chlorine is used under currently approved field conditions (5 mg/l free residual after 30 minutes), it is likely to effectively inactivate HAV and other enteroviruses.

Inactivation of all three test viruses by Army iodine was rapid at pH 9.5 (T-99.99 <10 minutes) under all conditions tested, and relatively slow at pH 4.5 and 5°C. For polio 1 and echo 1, 99.99% inactivation was not achieved in 60 minutes by 1 tablet per quart at 5°C and pH 7.0 and 4.5, or by 2 tablets per quart at 5°C and pH 4.5. The three strains of HAV tested were quite similar in response to iodine. Virus inactivation by iodine was generally more effective at higher pH, in cleaner water, at higher temperature and at higher iodine dose. However, the results indicate that Army iodine may not inactivate HAV and other enteroviruses efficiently under some conditions likely to be encountered in the field.

FOREWARD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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I. INTRODUCTION AND BACKGROUND

A. Importance of Hepatitis A Virus in Water

Hepatitis A or infectious hepatitis continues to be an important waterborne viral disease. HAV is an important waterborne enteric virus because of (i) the severity of the disease it causes, (ii) the high levels and prolonged time periods of its fecal excretion by infected persons, (iii) its considerable stability in water and wastewater, and (iv) the well documented evidence that it causes drinking waterborne disease (Purcell et al., 1984; Sobsey et al., 1988a). A recent compilation of reported outbreaks of waterborne disease in the United States listed a total of 23 outbreaks involving 737 cases of hepatitis A for the period 1971-1985 (Craun, 1988). In community water supplies most outbreaks of waterborne infectious disease, including outbreaks of hepatitis A, are caused by post-treatment contamination of finished waters in distribution systems (Lippy and Waltrip, 1984). In small and individual water supplies most outbreaks of hepatitis A and other infectious diseases are caused by lack of, interruption of, or inadequate treatment, including disinfection (Lippy and Waltrip, 1984; Craun, 1988). These epidemiological data demonstrate the importance of adequate treatment, and especially adequate disinfection, to prevent waterborne transmission of hepatitis A and other infectious diseases.

Recent reports suggest that HAV may be more resistant to various chemical and physical agents (Siegl et al., 1984) and more stable under various environmental conditions (Sobsey et al., 1986; 1988a) than other viruses and bacteria. Conventional water treatment practices utilizing chemical disinfection, primarily chlorination, are generally believed to be effective in producing microbiologically safe drinking water. However, the growing number of reports on the isolation of viruses from treated drinking water (Bitton et al., 1986) and the recent epidemiological evidence indicating that about 1/3rd of endemic, community-wide enteric illness of presumed viral etiology may be caused by conventionally treated drinking water (Payment et al., 1990) suggest that some viruses may survive treatment under certain conditions. The establishment of reliable water treatment practices and water quality standards to insure the virological safety of water supplies can be achieved only by fully understanding the response of HAV to water disinfectants such as chlorine and iodine.

B. Previous Studies on Disinfection of HAV in Water

Despite the need to determine the kinetics and extent of HAV inactivation by water disinfectants, relatively few studies on inactivation of HAV in water by chlorine have been reported and they provide conflicting information. No studies on the inactivation of HAV in water by iodine have been reported. Early HAV chlorination studies by Neefe et al. (1945, 1947) provided

indirect evidence that HAV is insensitive to combined chlorine. Using human volunteers for virus infectivity assay, Neefe and co-workers found that a total chlorine residual of 1 mg/l did not completely inactivate HAV in dilute fecal suspensions after a contact time of 30 minutes. The addition of sufficient chlorine to produce total and free chlorine concentrations of 1.1 and 0.4 mg/l, respectively, in purified effluent was required to prevent clinical infectious hepatitis in volunteers. Peterson et al. (1983) used marmosets to assay for HAV infectivity after chlorination of a partially purified preparation of HAV. The infectivity of the preparation, which contained about 1500 infectious units/ml, was only partially reduced by treatment with up to 1.5 mg/l of free residual chlorine at neutral pH for 30 minutes. These results, along with observations made during an outbreak of hepatitis A from a chlorinated groundwater supply in Georgetown, Texas (Hejkal et al., 1982), suggest that HAV is more resistant to conventional water chlorination processes than other enteroviruses and indicator bacteria.

In contrast to the findings of Neefe and co-workers, Peterson et al. and Hejkal et al., results of studies by Grabow et al. (1983) indicated that HAV may be more sensitive to free chlorine than previous studies and epidemiological evidence have suggested. Using serological techniques for assay of HAV infectivity in cell culture, Grabow and co-workers found that HAV was very sensitive to low levels of free chlorine relative to selected indicator viruses and bacteria. However, other studies by this group indicated that HAV was relatively resistant to combined forms of chlorine (Grabow et al., 1984). Recently, Sobsey et al. (1988b) found that purified, monodispersed HAV was inactivated rapidly by 0.5 mg/l free chlorine at 5°C, with times for 99.99% inactivation of <8 minutes between pH 6 and 9 and about 50 minutes at pH 10. However, HAV was found to be relatively resistant to a 10 mg/l dose of monochloramine, with a time for 99.99% inactivation of 117 minutes. In the same study free chlorine inactivation of coliphage MS-2 was generally similar to that of HAV but coxsackievirus B5 (CB5) was considerably more resistant than HAV. For monochloramine, inactivation of MS-2 was slower than that of HAV while CB5 inactivation was similar to that of HAV. Overall, The results of Sobsey et al. (1988b) for inactivation of HAV by free and combined chlorine are similar to those of Grabow et al. (1983) but different from (more rapid than) those of Peterson et al. (1983).

C. Military Need for Virus Disinfection of Water

In the military there is a special need for disinfectants that will be effective in destroying waterborne pathogens under adverse or emergency conditions, particularly when the quality of the water available for consumption is poor. Since World War II, the Armed Forces of this country have relied primarily on globaline tablets, an iodine-based disinfectant consisting of tetraglycine hydroperiodide, for disinfection of canteen water

and other small-volume field water supplies (O'Conner and Kapoor, 1970). Relatively little is known about the adequacy of this disinfectant in preventing the transmission of viral pathogens such as HAV in waters with varied physical and chemical characteristics. Outbreaks of infectious hepatitis associated with military activities have continued to occur since the development of globaline (Bancroft and Lemon, 1984). Although the effectiveness of globaline and other forms of iodine against HAV has not been evaluated, disinfection studies on their effectiveness for other enteric viruses, enteric bacteria and protozoan cysts have been reported (Safe Drinking Water Committee, 1980; Alvarez and O'Brien, 1982). However, given the substantial differences in the response of different enteric viruses to chlorine and other disinfectants (Liu et al., 1971; Sobsey, 1989), it is impossible to predict the efficacy of globaline or other disinfectants for inactivation of HAV in water.

In view of the limited data on HAV disinfection in general and the inconsistent findings of the few studies on HAV disinfection by chlorine, a critical evaluation of HAV inactivation by free and combined forms of chlorine and by iodine (globaline) is clearly warranted. Water quality variables, such as the presence of suspended inorganic particulates and soluble and colloidal organic matter, are important factors that need to be evaluated for their effect on the efficiency of disinfection of HAV. The adsorption of viruses to particulate matter in water has been well documented (Goyal and Gerba, 1979; Hurst et al., 1980; Schaub and Sagik, 1975; Sobsey et al., 1980). Particulates may protect viruses in the aqueous environment by sheltering viruses from disinfectant exposure or by consuming or chemically changing the disinfectant. Naturally-occurring soluble and colloidal organic matter (such as humic and fulvic acids) in natural waters, finished waters and wastewaters may also be a factor in reducing the efficacy of the disinfection process by consuming or changing the active species of the chemical agent. The effects of suspended matter and soluble and colloidal organic matter on disinfection of HAV and other viruses by chlorine and iodine have not been adequately addressed by the few studies reported in the literature.

The study of HAV inactivation kinetics by chlorine and iodine is now feasible with the utilization of new methodologies for the cultivation and enumeration of HAV in cell cultures (Cromeans et al., 1987; 1989; Daemer et al., 1981; Frosner et al., 1979; Lemon et al., 1983; Provost and Hilleman, 1979). The focus of this project is to examine the kinetics and extent of HAV inactivation by chlorine and iodine, with special emphasis on determining the influence of important water quality variables on chlorine and iodine inactivation of HAV and, for comparison, selected model viruses. This report presents research findings obtained over the entire course of this project.

II. STATEMENT OF THE PROBLEM AND OBJECTIVES

A. Statement of the Problem

The problem to be studied is the efficiency (kinetics and extent) of inactivation of hepatitis A virus (HAV) by chlorine (calcium hypochlorite) and Army iodine (globaline) in waters of different quality. In these experiments the inactivation of other viruses or of different strains of HAV is compared. Studies on chlorine and iodine inactivation of HAV and other enteroviruses were done to determine if HAV is relatively resistant or sensitive to these disinfectants compared to other enteroviruses. The inactivation of several strains of HAV by chlorine and iodine was compared to determine whether or not different HAV strains were appreciably different in their responses to these disinfectants.

B. Specific Ojectives

The specific objectives of this study are to determine the kinetics and extent of inactivation of HAV (strain HM175), poliovirus 1 (strain LSc) and echovirus 1 (strain V239) by free chlorine (hypochlorite ion/hypochlorous acid) and by iodine (globaline tablets) in waters of different quality. As an alternative to using highly purified, monodispersed virus preparations, disinfection experiments utilize partially purified, aggregated preparations in order to better model the physical state of the viruses in natural aquatic environments.

The water quality variables to be studied are water type, pH and temperature because all of these factors greatly influence the effectiveness of microbial inactivation by chemical disinfectants (Sobsey, 1989). The two water qualities are buffered halogen demand-free (BHDF) water and so-called worst case water consisting of BHDF water containing 5 NTU of bentonite clay turbidity and 10 mg/l of a 1:1 (W/W) mixture of humic and fulvic acids. Worst case water contains both clay particulates and soluble and colloidal organic acids because these are major water quality factors influencing the effectiveness of microbial inactivation by chemical disinfectants. The pH levels to be studied are pH 4.5, 7.0 and 9.5, because this represents the range of pH levels likely to be encountered in drinking waters. The temperatures to be studied are 5° and 25°C, which covers most of the temperature range that may be found in drinking waters. The comparative inactivation of three strains of HAV (HM175, MD-1 and CR326) by free chlorine and iodine was determined for selected conditions, namely, the lowest dose of disinfectant, the poorer water quality, the lower temperature and the pH extremes.

Waters are seeded with a mixed virus preparation containing sufficient quantities of HAV, poliovirus 1, and echovirus 1 to follow the inactivation of each virus over at least 4 $\log_{10}$ units (99.99%). The latter two viruses are included in order to

compare HAV sensitivity to halogen disinfectants to that of other viruses which have been previously studied in this regard.

It should be noted that some of the objectives of this study have been modified from those originally planned due to technical limitations, constraints arising from limitations of resources, and redundancy to other experiments. Specifically, experiments using 3.6 mg/l iodine, groundwater only, clay turbidity only, organic acids only, MS2 bacteriophage and Escherichia coli have been eliminated.

III. METHODS AND MATERIALS

A. Viruses, Cell Cultures and Virus Purification

1. HAV. The HM175 (NIH prototype) strain of HAV, originally isolated from feces of an infected human in Australia (Daemer et al., 1981; Lemon et al., 1983; Gust et al., 1985) was produced in persistently infected BS-C-1 cells grown in 850 cm^2 roller bottles or 6000 cm^2, ten-tiered cell factories (Inter Med, A/S NUNC, Roskilde, Denmark) or by acute, serial infection of FRhK-4 (fetal rhesus kidney-derived continuous line) cells (Cromeans et al., 1987) incubated at 37°C. Prior to persistent infection of BS-C-1 cells, the virus had been serially passaged 6 times in marmosets, 10 times in primary African green monkey kidney (AGMK) cells and 7 times in BS-C-1 cells. The passage history of HAV HM175 grown in FRhK-4 cells is described elsewhere (Cromeans et al., 1987; 1989). The MD-1 strain of HAV, originally isolated from contaminated groundwater implicated in a community-wide outbreak of hepatitis A (Sobsey et al., 1984), was produced in persistently infected A549 (human lung carcinoma) cells grown in roller bottles. This strain of HAV was originally passed 3 times in secondary African green monkey kidney cells prior to passage in A549 cells. The CR326 strain of HAV was isolated from the stool of an infected individual in Costa Rica by passage in marmosets and subsequent propagation in marmoset liver explants and then fetal rhesus kidney cell cultures (Provost and Hilleman, 1979). HAV strain CR326 of unspecified passage number in FRhK-4 cells was obtained from the Hepatitis Laboratories, Centers for Disease Control, Atlanta, Ga. In this laboratory it was passaged once in A549 cells and then propagated in persistently infected FRhK-4 cells.

Infectivity of HAV strain HM175 was assayed by radioimmunofocus assay (RIFA) in BS-C-1 cells or plaque assay in FRhK-4 cells (Lemon et al., 1983; Sobsey at al., 1985; Cromeans et al., 1987). HAV strains MD-1 and CR326 were assayed for infectivity by RIFA in A549 and FRhK-4 cells, respectively. The RIFA is an enumerative assay analogous to a plaque assay, except that non-cytopathic, focal areas of infected cells are visualized by an immune autoradiographic method. Incubation periods for RIFA and

plaque assay ranged from 1-2 weeks, depending on the strain and passage level of the virus.

For acute infection of host cells, HAV was inoculated into newly confluent cell layers in roller bottles at a multiplicity of 0.01-0.1 radioimmunofocus forming units (RFU) or plaque forming units (PFU) per cell. After 1-2 weeks of infection, HAV was harvested from infected cells by freezing and thawing three times, sonicating for 1 minute using a probe-type sonicator, homogenizing with an equal volume of trichlorotrifluoroethane at high speed for 2 minutes in an Omni mixer (Omni International, Waterbury, Connecticut), centrifuging at 4,000 x g for 10-20 minutes, and recovering the virus-containing supernatant fluid.

Persistently infected cells were passaged every two to four weeks by trypsinization and then resuspension of some of the cells in growth medium at a concentration of about 1 x 10^5 cells/ml for re-inoculation into culture vessels. At each passage, some of the persistently infected cells and all of the culture fluids were harvested as crude virus stock. Harvested infected cells were centrifuged at low speed (about 3000 x g), resuspended in small volumes of phosphate-buffered saline (PBS), pH 7.5, and extracted with an equal volume of chloroform. The HAV-containing PBS was recovered by low speed centrifugation to remove cell debris and chloroform. The cell debris and chloroform was extracted four to six more times with equal volumes of PBS to obtain additional virus, and all PBS extracts were pooled as virus stock. HAV in cell culture fluids was concentrated by precipitation with polyethylene glycol (PEG) 6000 (12% w/v, pH 7.2) overnight at 4°C. Resulting precipitates were recovered by low speed centrifugation, resuspended in a small volume of PBS and extracted with a volume of chloroform equal to the PBS volume in order to remove excess PEG. The PBS extracts were cleared of chloroform and PEG by low speed centrifugation.

PBS extracts of cells and PEG concentrates from culture fluids were pooled, and HAV was pelleted by ultracentrifugation at 30,000 RPM (105,000 x g) for 4 hours at 5°C. Resulting pellets were resuspended in small volumes of 0.05M phosphate-buffered distilled water (PBDW) and supplemented with CsCl to give a density of 1.33 g/ml. These samples were ultracentrifuged to equilibrium in self-generated gradients at 25,000 RPM (90,000 x g) and 5°C for 3 days using the SW27 rotor (Beckman Instruments). Gradients were harvested in fractions from the bottoms of the tubes and assayed for HAV infectivity by RIFA or plaque assay. Fractions with the peak of HAV infectivity were desalted by untrafiltration and washing with PBDW using Centricon 30 tubes (Amicon Inc). Desalted fractions were layered onto 10-30% sucrose gradients in phosphate buffered halogen demand-free water, pH 7.5, (PBDFW) and subjected to rate zonal centrifugation in the SW27 rotor at 25,000 RPM (90,000 x g) and 5°C for 5.5 hours. Under these conditions, single virions would sediment about 2/3rds of the distance from the top to the bottom of the

tube. Gradient fractions were harvested from the top of the tube and assayed for HAV infectivity by RIFA or plaque assay. Gradient fractions were characterized as containing single virions or small, medium or large aggregates of HAV according to their position in the gradient. HAV gradient fractions were then pooled and in most experiments mixed with appropriate amounts of gradient fractions of the other test viruses such that the total amount of each virus consisted of about 8% single virions, 19% small aggregates, 39% medium aggregates and 34% large aggregates. The titer of each virus in the mixture was 1-5 x 10^8 infectious units/ml. For experiments on the inactivation of different strains of HAV by chlorine and iodine, pools of HAV gradient fractions containing the previously specified distribution of singles and different sized aggregates with titers of 1-5 x 10^8 infectious units/ml were tested directly. These virus stocks and mixtures were further diluted 1:5 in halogen demand-free water for use in disinfection experiments in order to reduce halogen demand.

2. Echovirus 1 and Poliovirus 1. Echovirus 1 (strain V239) and poliovirus 1 (strain LSc) were grown and assayed by the plaque technique in BGM (African green monkey kidney-derived) and MA104 (rhesus monkey kidney-derived) continuous cell lines, respectively, as previously described (Sobsey et al, 1978). In order to assay each animal virus type (HAV, poliovirus and echovirus) in samples containing all three viruses, the other two viruses were neutralized by adding antibodies (antisera) against them to the virus diluent. Antisera were reference reagents prepared for the National Institutes of Health, and they were obtained from the American Type Culture Collection, Rockville, Maryland. For example, poliovirus was assayed by neutralizing echovirus type 1 using antiserum against echovirus type 1 in the poliovirus diluent. HAV did not have to be neutralized in assays for poliovirus or echovirus because it was non-cytopathic, grew slowly and did not interfere with the assays for these other two viruses (unpublished results).

Poliovirus and echovirus were first plaque-purified 2-3 times and then grown in large quantities under either one-step growth conditions (>5 PFU/cell) or at low multiplicity of infection (MOI; 0.01-0.1 PFU per cell). Initially, viruses were grown under one-step conditions and harvested prior to cell lysis in the hopes of obtaining greater proportions of viruses in aggregated form than when grown at low MOI and harvested after cell lysis. However, the proportion of aggregated viruses did not differ for the two growth methods. Therefore, low MOI conditions with harvest after cell lysis was used in later experiments because it required less virus to infect and provided greater flexibility for harvest time.

Crude virus stocks were harvested from infected cells at 5-7 hours post-infection under one-step growth conditions or from infected cell lysates several days post-infection at low MOI when

cytopathic effects were 4+. Virus was liberated from cells and cell debris by freezing and thawing, and then cell debris was removed by centrifugation at low speed (10,000 x g for 15-30 minutes). Viruses in resulting supernatants were pelleted by ultracentrifugation (105,000 x g and 5°C for 4 hours). Resulting virus pellets were resuspended in buffered HDFW, homogenized 1 minute at top speed in an Omni Mixer, and in some cases centrifuged at 10,000 x g and 5°C for 20 minutes to remove additional debris. After supplementing the sample with CsCl to give a density of 1.33 g/ml, viruses were banded to equilibrium as for HAV. Gradient fractions were harvested and assayed for virus infectivity, and virus peak fractions were desalted using Centricon 30 ultrafiltration units. These fractions were pooled and subjected to rate-zonal centrifugation in 5% (or 10%) to 30% sucrose gradients as for HAV. Gradient fractions were harvested and assayed for virus infectivity and appropriate amounts of virus fractions were added to HAV samples to give the desired distribution and virus titers of single virions as well as small, medium and large aggregates.

B. Glassware, Test Waters and Halogen Reagents.

All glassware for disinfection experiments and preparation of halogen demand-free (HDF) virus stocks was soaked at least 4 hours in a strong chlorine (10-50 mg/l) solution and then rinsed thoroughly with halogen demand-free water (HDFW) prior to use. HDFW and buffer solutions for disinfection experiments were prepared from glass-distilled, deionized water by adding chlorine to approximately 10 mg/l. After storage at room temperature for at least 1/2 day, water or buffers were dechlorinated by exposure to a submersible ultraviolet light. HDF, phosphate-based buffers, 0.01M, were used to prepare halogen test solutions and buffered water for disinfection experiments.

Two types of water were used in disinfection experiments: 0.01M phosphate buffers in halogen demand free water (PBDFW) and "worst case (WC) water". Worst case water was made by supplementing halogen demand free, phosphate buffers with a 1:1 mixture (W/W) of humic and fulvic acids to a final concentration of 10 mg/l and with 5 nephlometric turbidity units (NTU) of bentonite clay. Stock humic and fulvic acids were purified and concentrated from the highly colored water of Bay Tree Lake as previously described (Sobsey and Hickey, 1985). Wyoming bentonite was sized fractioned, washed and prepared as a concentrated stock suspension for addition to test waters as previously described (Cromeans and Sobsey, 1985).

Reagent grade calcium hypochlorite was used to prepare solutions of hypochlorous acid (HOCl) at pH 4.5, predominantly hypochlorite ion (OCl^-) at pH 9.5, and mixtures of these free chlorine species at pH 7.0. Hypochlorite stock solutions of about 100 mg/l were prepared by dissolving about 0.2 g of $Ca(OCl)_2$ in 1 liter of

HDFW. Stock solution was then diluted in test water (halogen demand-free, 0.01M phosphate buffer or "worst case water," pH 4.5, 7.0 or 9.5) to give the target chlorine concentration. Target chlorine concentration was verified by chemical analysis.

Iodine solutions were prepared from globaline tablets, which contain tetraglycine hydroperiodide as the active ingredient and disodium dihydrogen pyrophosphate as a buffer. Globaline concentrations to be tested were the two concentrations based upon recommended Army field use: 1 to 2 tablets per quart, giving concentrations of about 8 and 16 mg of titratable iodine per liter, respectively. Iodine solutions were prepared by dissolving 1 or 2 globaline tablets in about 900 ml of 0.01M phosphate buffered HDFW or worst case water and adjusting to pH 4.5, 7.0 or 9.5 with NaOH or H_2SO_4. These samples were brought to a volume of 927 ml with HDFW and iodine concentration was measured. The volume of iodine solution was such that addition of 1 part of test virus mixture to 9 parts of iodine solution would dilute the iodine to the target concentration. To be used for experiments, samples with 1 or 2 tablets per quart had to have initially measured iodine concentrations of >7.5 and >15 mg/l, respectively.

C. Halogen Analysis.

Iodine and chlorine concentrations were measured by DPD colorimetric methods as described in Standard Methods for the Examination of Water and Wastewater, 16th edition (American Public Health Association, 1985). Standardization of procedures for chlorine measurement was by the DPD ferrous titration method, and for iodine measurement by using potassium bi-iodate as a primary standard.

D. Protocols for Disinfection Experiments.

Experiments on virus disinfection by free chlorine or Army iodine in buffered HDFW or worst case water, pH 4.5, 7.0 and 9.5, were done according to the flow diagram shown in Figure 1. Samples were in 16 mm diameter x 100 mm long (or 25 mm diameter x 125 mm long) test tubes containing Teflon coated, magnetic stir bars. They were placed in a water bath to maintain a temperature of 5 or 25°C and magnetically mixed. For experiments with free chlorine, at concentrations of 1 and 5 mg/l in PBDFW, at concentrations of 3 and 7 mg/l in worst case water or iodine at doses of 1 and 2 tablets per quart (initial iodine concentrations of >7.5 mg/l and >15 mg/l, respectively), 0.85 ml of purified HAV strain (HM175, MD-1 or CR326) or virus stock mixture (HAV, polio and echo), diluted 1:5 in HDFW, was added to 7.65 ml of a chlorine solution containing 1.1 or 5.5 mg/l free chlorine in PBDFW, 3.0 or 7.0 mg/l free chlorine in WC water, or >7.5 or >15 mg/l iodine in PBDFW or WC water and briefly mixed. A second test tube containing only chlorine or iodine solution in test water served as a halogen control. A third tube containing a

1:10 dilution of stock virus in test water served as a virus control. Samples of 0.7 ml were withdrawn from the reaction tube (halogen solution plus added virus) for viral analysis at 0.33, 1.0, 3.0, 10, 30 and 60 minutes after virus addition. These samples were diluted two-fold immediately in virus diluent (2X Eagle's MEM) containing 1% $Na_2S_2O_3$. A further five-fold dilution (10-fold overall) was made, followed by serial 10-fold dilutions made in separate diluents for each virus. These dilutions were stored at 4°C for subsequent virus assay. After the 60 minute reaction period, the remaining reaction mixture (halogen plus added virus) and the halogen control sample (halogen only) were re-analyzed for free and combined chlorine or iodine. In later experiments, halogen residuals were also measured after only 30 minutes of reaction as well. Samples from the virus control tube (virus plus test water) were diluted serially 10-fold at the beginning and the end of the 60 minute reaction period for subsequent virus assay.

E. Experiments on Halogen Stability in Mock Samples

In order to better characterize the rate and extent of halogen loss over time in test samples, a series of experiments were done in which 9 parts of halogen solution at 1.1 times the desired concentration was mixed with 1 part of a "mock" purified virus preparation consisting of 23% sucrose in 0.01M phosphate buffered HDF water. Samples of the mixture were analyzed for residual halogen using the methods described above at various times over a 60 minute period. Experiments were done at pH 4.5, 7.0 and 9.5 in PBDFW using iodine at a dose of 1 tablet per quart and chlorine at doses of 1 and 5 mg/l and in WC water using iodine at 1 and 2 tablets per quart and chlorine at doses of 6.5 and 3.0 mg/l in WC water. Temperatures of halogen stability experiments were done at either 5°C only or 5 and 25°C, depending on halogen and water quality.

F. Determination of Viral Aggregates: Single Particle Approximation (SPA)

The degree of viral aggregation in phosphate HDFW and worst case water was determined by the single particle approximation (SPA) test method as described by Floyd and Sharp (1977). This method was also used to determine virus interaction with humic and fulvic acids, and Wyoming bentonite clay in the worst case water experiments.

The SPA test was used to determine the fraction of the total PFU or RFU of a sample that was attributed to single virions. This was done by isolating the region of the ultracentrifuge tube between the slowest of the singles and the slowest of the small aggregates (pairs) (see Figure 2). The top half (4.14 cm) of the tube should contain single virions and the slowest of the small viral aggregates, and the bottom half should contain small, medium, and large viral aggregates. The percent singles of a

virus fraction was then determined by comparing the PFU titer of the top half of the tube with that of the whole mixture.

HAV, poliovirus 1, and echovirus 1 sucrose gradient fractions that were thought to contain either single virions, small, medium, or large viral aggregates were used in the SPA test. The determination of these categories of viral aggregation was based on the relative positions of virus fractions in a sucrose gradient profile obtained during virus purification. The single virion peak sample constituted the first and usually the predominant peak of viruses first encountered in the gradient starting from the top of the tube. The remaining fractions of the gradient extending to the bottom of the tube were then divided into three groups or thirds. The first group past the singles peak was designated small aggregates, the next group was designated medium aggregates and the last group (closest to the bottom of the tube) was desiganted large aggregates.

These virus fraction classes were suspended in the following test waters: halogen demand free 0.01 M potassium phosphate buffer at pH 4.5, 7.0, and 9.5 and potassium phosphate buffer containing 10 mg/L humic and fulvic acids and Wyoming bentonite clay at 5 NTU at pH 4.5, 7.0, and 9.5. The virus sucrose gradient fractions containing approximately 1 x 10^7 PFU were diluted 1 to 24 in each test water and mixed thoroughly. A sample was removed for assay to determine the titer of the whole virus mixture.

Experiments were run in a SW 41 Ti swinging bucket rotor at 40,000 rpm without brake for 19.6 min at 20C in a Beckman Model LS-40 ultracentrifuge. The acceleration and deceleration times must be considered as part of the run time. If both these operations were done with constant acceleration and deceleration then their total contribution to the sedimentation rate was equal to one third of the total time involved and this was known as the Tc or control time. The Tc time was subtracted from the 19.6 min run at 40K to give the actual run time at full speed. (personal communication, D.G. Sharp, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill). "Dry runs" using tubes filled with water were done to determine the average time up to top speed and the average time down to stop. The average acceleration time for the fully loaded Ti 41 swinging bucket rotor was 6.0 min. The average deceleration time was 34.25 min. This gave a total of 40.25 min up and down time. Therefore, one third of the 40.25 min total time equaled 13.42 min, and this was subtracted from 19.6 min to give a total run time at full speed of 6.18 min. With each ultracentrifuge run, a PBS virus control was also included. This control contained known single virus particles of either HAV, poliovirus 1, or echovirus 1 suspended in PBS at pH 7.5. When the run was completed, the centrifuge tubes were carefully removed from the rotor. The liquid was removed from the top half of each tube (the top 4.14 cm of liquid in the tube). A special apparatus was

designed to remove liquid from only the top half of the tube with minimum disturbance of the tube contents (Figure 3).

The top half of the contents of each centrifuge tube was then assayed. Since only the top half of each tube was removed to be assayed, the singles fraction of each sample was diluted by a factor of 2.978. The PFU/ml of the top half of the tube was multiplied by this dilution factor and the product was divided by the PFU/ml of the whole mixture to give the approximate fraction of the PFU that were produced by singles.

G. Data Analysis

Results of the disinfection assays were plotted on semi-log paper as the logarithm of the surviving fraction, $\log_{10} N_t/N_o$, versus time, t, in accordance with Chick's Law of Disinfection, where N_o = the average of virus titer (in control tubes) at 0 time and either 30 or 60 min, N_t = virus titer at time t, and t = contact time. The data are also presented in tabular form (see Appendix Tables) as both virus titers (PFU or RFU per ml) and surviving virus over time as N_t/N_o and $\log_{10} N_t/N_o$.

The times required for 99.99% inactivation of initial viruses, T-99.99 (4 $\log_{10}$ reduction) were determined by linear regression analysis and either interpolation or extrapolation to T-99.99 levels, as required. In some cases detection limit values for virus infectivity had to be used to determine these values. The detection limit of an assay system represents the limit of that assay's ability to detect virus in samples tested. Detection limit points were obtained by assuming that at least 1 PFU/volume of the least dilute dilution assayed could still be present at the time point beyond which virus was last seen. When detection limit points were used to calculate T-99.99 values, they are designated by a less than symbol (<). A greater than symbol (>) indicates that 99.99% virus reduction was not achieved during the 60 min experiment.

Statistical analysis of the data for virus inactivation by free chlorine was done by the Kruskal-Wallis one way analysis of variance test. This test is a nonparametric analysis of variance of ranked data. It substitutes an approximation to a Chi-square for a F statistic (Conover, 1971; Daniel, 1978). The data for each virus from each experiment were ranked using the detection limit time point (0.33, 1, 3, 10, 30, 60, and > 60 min). Subsequently, the data were analysized by the Kruskal-Wallis test run in the Systat program package (SYSTAT Inc., Evanston, IL). The level of significance for all analysis was set at $p = 0.1$ (Wilkeninson, 1988). In addition, the effect of disinfectant concentration on the rates of virus inactivation and the sensitivities of each virus to free chlorine were determined by the Watson time concentration (CT) relationship or the Van't Hoff equation (Hiatt, 1964; Hoff, 1986).

The form of the relationship used was:

$$K = C^{n}T$$

where

K = the constant for a specific microorganism exposed under certain conditions,
C = disinfectant concentration,
n = the constant called the coefficient of dilution, and
T = the contact time required for a certain unit of inactivation

The disinfectant concentrations (C) used were the initial chlorine concentrations, except in cases where virus survival was 10 minutes or longer, in which case the concentration was calculated as an average of the beginning and ending chlorine concentrations.

The coefficient of dilution (n) according to Van't Hoff is a measure of the order of the reaction and is obtained by plotting disinfection concentration versus contact time on double logarithmic paper. When $n > 1$, disinfection efficiency decreases rapidly as the disinfectant is diluted; here disinfectant concentration is more important than contact time. When $n < 1$, the contact time is more important than disinfectant concentration. When $n = 1$, concentration and time are of equal importance (Hoff, 1986).

IV. RESULTS AND DISCUSSION

A. Inactivation of HAV (HM175), Poliovirus 1 and Echovirus 1 by Free Chlorine in Buffered HDFW.

The mean results of duplicate experiments on inactivation of HAV (strain HM175), poliovirus 1 and echovirus 1 by 1 and 5 mg/l doses of free chlorine at 5 and 25°C and pH 4.5, 7.0 and 9.5 are summarized in Tables 1 and 2 as times for 99.99% virus inactivation (T-99.99) and in Figures 4-9, where log N_t/N_o is plotted versus contact time. The results of individual experiments are given in Appendix Tables A1-A12. At a chlorine dose of 1 mg/l, HAV was inactivated rapidly at all pH levels tested, with T-99.99 values of <8 minutes (Table 1; Figures 4-6). As expected, HAV inactivation was more rapid at 25°C than at 5°C, and in most cases the inactivation rate was 2-3 times faster at the higher temperature. It is interesting to note that the rate of HAV inactivation was most rapid at pH 7.0 but not much longer at pH 9.5 than at pH 4.5. This suggests that HAV is either relatively sensitive to hypochlorite ion (the predominant chlorine species at pH 9.5), that it is highly sensitive to the very low concentration of hypochlorous acid that is present at pH 9.5, and/or that the state of virus aggregation differed at different pH levels and may have influenced HAV inactivation kinetics.

Compared to HAV, both poliovirus 1 and echovirus 1 were more resistant to free chlorine, at least at pH 9.5. In general, echo 1 was inactivated more slowly than polio 1. Both polio 1 and echo 1 were inactivated slowly by a 1 mg/l dose of free chlorine at pH 9.5 and 5°C, with T-99.99 values of 59 and >>60 minutes, respectively. These results are consistent with those of previous studies showing that HAV and other enteroviruses are inactivated more slowly at higher pH levels where hypochlorite ion predominates (Engelbrecht et al., 1980; Scarpino, 1979; Sobsey et al., 1988b). At 25°C and pH 9.5, inactivation rates of both polio 1 and echo 1 were considerably faster than at 5°C (T-99.99 = 8.2 and 30 minutes, respectively). Both polio 1 and echo 1 were inactivated relatively rapidly at pH 7.0 and 4.5, where greater proportions of the more virucidal hypochlorous acid are present, than at pH 9.5. At these pH levels, T-99.99 values for polio 1 and echo 1 were <7 minutes. As observed for HAV, inactivation of polio 1 and echo 1 was consistently greater at 25°C than at 5°C.

Data for HAV (HM175), polio 1 and echo 1 inactivation by a 5 mg/l dose of free chlorine in phosphate buffered DFW at 5 and 25°C are shown in Figures 7-9 and summarized in Table 2 as T-99.99 values. HAV was inactivated rapidly by a 5 mg/l dose of free chlorine, with T-99.99 values <3 minutes, and HAV inactivation was greater at 25 than at 5°C. As previously noted for a 1 mg/l dose of free chlorine, HAV inactivation by a 5 mg/l dose of free chlorine was more rapid at pH 7.0 than at pH 4.5 or 9.5 (at least at 5°C), and

HAV inactivation times at pH 9.5 were not much longer than those at lower pH levels.

At a 5 mg/l dose of free chlorine and pH 9.5, both polio 1 and echo 1 were inactivated more slowly than HAV. However, at pH 4.5 and 7.0, all three viruses were inactivated very rapidly, with T-99.99 values of <2 minutes. Comparison of T-99.99 values of Tables 1 and 2 indicates that all three viruses were generally inactivated more rapidly by 5 mg/l free chlorine than by 1 mg/l free chlorine, as would be expected. A 5 mg/l free chlorine concentration and 30 minute contact time are Army standard operating conditions for chlorine disinfection of field water supplies. Therefore, it is important to note that in PBDFW HAV and the other two enteroviruses tested (polio 1 and echo 1) were substantially (>99.99%) inactivated under these conditions, with the exception of echovirus 1 at pH 9.5 and 5°C, which required and estimated 33 minutes for 99.99% virus inactivation. These results suggest that enteroviruses will be adequately controlled by present Army conditions for chlorine disinfection of water, at least in the absence of excessive turbidity, halogen demand or other interferences.

B. Inactivation of HAV (HM175), Poliovirus 1 and Echovirus 1 by Free Chlorine in Worst Case Water

The mean results of replicate experiments on inactivation of HAV (strain HM175), poliovirus 1 and echovirus 1 by 3 and 7 mg/l doses of free chlorine in worst case water (phosphate buffered HDFW containing 10 mg/l humic and fulvic acids and 5 NTU bentionite clay turbidity) at 5 and 25°C and pH 4.5, 7.0 and 9.5, are summarized in Tables 3 and 4 as times for 99.99% virus inactivation and in Figures 10-15 where log N_t/N_o is plotted versus contact time. The results of individual experiments are given in Appendix Tables A13-A24. At a free chlorine dose of 3 mg/l, HAV was inactivated rapidly, with T-99.99 values of <1 minute (Table 3; Figures 10-12). As noted for experiments using 1 and 5 mg/l of free chlorine in PBDFW, the rate of HAV inactivation was not slower at pH 9.5 than at the lower pH levels of 7.0 and 4.5. This again suggests that either HAV is relatively sensitive to hypochlorite ion or very low concentrations of hypochlororous acid or that there were differences in the extent of virus aggregation at the different pH levels. An obvious temperature effect on HAV inactivation was not observed, as HAV was inactivated so rapidly at even 5°C that T-99.99 values were similar and very low (no more than 0.6 minutes) at both temperatures.

Compared to HAV, both poliovirus 1 and echovirus 1 were generally more resistant to a 3 mg/l dose of free chlorine in worst case water. In general, echovirus 1 was inactivated less rapidly than either poliovirus 1 or HAV. With the exception of echovirus 1 at pH 9.5 and 5°C, both poliovirus 1 and echovirus 1 were inactivated rapidly by a 3 mg/l dose of free chlorine at 5°C in

worst case water, with 99.99% virus reductions in <7 minutes. For echovirus 1 at pH 9.5 and 5°C, the T-99.99 value was 72 minutes. As previously observed in PBDFW, both poliovirus 1 and echovirus 1 were generally inactivated more slowly at pH 9.5, where hypochlorite ion predominates, than at the lower pH levels of 7.0 and 4.5, where the more virucidal hypochlorous acid predominates. At 25°C, all viruses were inactivated in less than 7 minutes. In general, both poliovirus 1 and echovirus 1 were inactivated more rapidly at 25°C than at 5°C for corresponding pH levels. These results again demonstrate the previously observed phenomenon of increased virus inactivation at higher temperature.

Data for HAV (HM175), poliovirus 1 and echovirus 1 inactivation by a 7 mg/l dose of free chlorine in worst case water at pH 4.5, 7.0 and 9.5 and 5 and 25°C are shown in Figures 13-15 and summarized in Table 4 as T-99.99 values. HAV was inactivated rapidly at this free chlorine dose, with T-99.99 values of 1.2 minutes or less. Both poliovirus 1 and echovirus 1 were also inactivated rapidly at this free chlorine dose, with T-99.99 values of <5 minutes. Thus regardless of pH and temperature, all three viruses were inactivated rapidly by a 7.0 mg/l dose of free chlorine in worst case water. However, as previously observed in PBDFW, poliovirus 1 and echovirus 1 were inactivated somewhat more slowly at pH 9.5 than at the two lower pH levels of 7.0 and 4.5 (at least at 5°C). In addition, poliovirus 1 and echovirus 1 were inactivated more rapidly at 25°C than at 5°C, at least at pH 9.5.

The results of these experiments indicate that HAV and other enteroviruses are capable of being adequately inactivated (by 99.99%) by present Army conditions for disinfection of water (5 mg/l residual after 30 minutes) even in the presence of excessive amounts of dissolved and colloidal organic matter and turbidity.

C. Stability of Free Chlorine in Mock Experimental Water

Because of concerns about the stability of free chlorine in test samples, mock virus inactivation experiments were done in which sucrose in PBDFW (mock virus stock) was added to chlorine solutions in PBDFW and worst case water at pH 4.5, 7.0 and 9.5 and 5 and 25°C. These solutions were sampled at various times over 30 or 60 minutes and analyzed for residual free chlorine. As shown by the results in Figure 16, free chlorine was quite stable in PBDFW under most of the conditions tested. Free chlorine losses in PBDFW at both 5 and 25 °C were <10% of the initial residual in 60 minutes at all three pH levels tested. Thus, neither pH nor temperature had an appreciable influence on chlorine stability in PBDFW.

Free chlorine stability experiments were also performed in worst case water at two free chlorine doses (about 3 and 6.5 mg/l), at three pH levels (4.5, 7.0 and 9.5) and at the lower temperature (5°C). Only the lower temperature was tested because experiments

with PBDFW indicated no appreciable temperature effect on chlorine stability. Furthermore, no appreciable difference was observed in free chlorine stability in actual disinfection experiments in worst case water at 5°C and 25°C. For comparison, free chlorine stability was re-tested in PBDFW at the lower chlorine dose of 3.0 mg/l. The results of experiments on stability of a 3.0 mg/l dose of free chlorine in PBDFW and WC water are summarized in Table 5; for a 6.5 mg/l dose of free chlorine in WC water, results are summarized in Figure 17. As shown by the results of experiments on stability of about 3.0 mg/l free chlorine in both WC and PBDF waters at 5°C, it can be seen that free chlorine was very stable in PBDFW, with about 90% of initial free chlorine persisting for 30 minutes. Free chlorine losses were greater in WC water, with 30 minute residuals of about 2.7, 2.0 and 2.2 mg/l at pH 4.5, 7.0 and 9.5, respectively. However, losses never exceeded 40% of the initial free chlorine. These results indicate that free chlorine stability in WC water was somewhat lower than that in PBDFW, with the greatest losses at pH 7.0 and 9.5. Most of the free chlorine loss occurred within the first 5 minutes of contact. The results of free chlorine stability experiments in WC water at a dose of 6.5 mg/l are summarized in Figure 17. It was observed that much of the initial free chlorine dose of about 6.5 mg/l persisted for 30 minutes, with free chlorine residuals of about 5.5, 4.25 and 4.5 mg/l at pH 4.5, 7.0 and 9.5, respectively.

Overall, free chlorine was relatively stable in mock experimental waters. These results suggest that the somewhat greater losses of free chlorine observed in some virus inactivation experiments were probably due to additional chlorine demand by the stock virus preparations used. Because of the goal to have free chlorine residuals of either 1 or 5 mg/l after 30 minutes of contact in virus inactivation experiments and the observed losses of free chlorine in stability experiments on worst case water, it was determined that initial free chlorine concentrations of 3 and 7 mg/l in worst case water would assure residuals of 1.0 and 5.0 mg/l, respectively.

D. Statistical Analyses of Data for Virus Inactivation by Free Chlorine

Statistical analysis on the effect of virus type, pH, temperature, chlorine concentration, and water quality on disinfection efficiency were performed. The Kruskal-Wallis one way analysis of variance was used to analyze the PBDFW and WC water inactivation data. If there are k populations of disinfection experiments containing n_1, $n_2 \ldots n_k$ observations, the total number of observations is denoted by n. That is:

$$n = \sum_{i=1}^{k} = n_i.$$

The null hypothesis is that the population medians are equal, so that the populations are identical. Each observation is replaced by its rank in the ordering of all samples together. In applying this test to the data of disinfection experiments, virus inactivation results at each pH, temperature, and chlorine concentration were ranked according to the contact time at which the virus detection limit was reached (the virus detection limit time point). Rank 1 was assigned to a virus sample with a detection limit point of 0.33 min, rank 2 to 1.0 min detection limit, and so on to 60 min. Tied observations were assigned the average of the ranks that would be assigned if there were no ties (see Appendix B, Table I).

Table II, Appendix B, summarize the results of the statistical analysis. The level of significance was set at $p = 0.1$. From these analyses, it was shown that chlorine dose, water quality, temperature and pH all had a significant effect on virus disinfection efficiency. Virus type did not have a significant effect on disinfection efficiency (Table II, $p = 0.372$). The lack of statistical significance for disinfection efficacy among virus types is interesting because there were discernable differences in virus inactivation rates in terms of T-99.99 values. The results of both PBHDFW and WC water disinfection experiments show that HAV was rapidly inactivated by free chlorine at all conditions tested with T-99.99 values ranging from <0.38 to 7.6 minutes. Poliovirus 1 was somewhat more resistant than HAV with T-99.99 values ranging from <0.4 to 57 minutes at all conditions tested. Echovirus 1 was the most resistant with T-99.99 values ranging from <0.31 to >>60 min.

Statistical analysis showed that chlorine concentration had a significant effect on virus disinfection efficiency (Table II, Appendix B). The results of PBDFW and WC water experiments revealed that increasing the chlorine concentration increased virus inactivation efficiency. For example, the T-99.99 values for HAV, poliovirus 1, and echovirus 1 were 7.6, 57, and >>60 minutes, respectively, in HDF water with 1.0 mg/L free chlorine at pH 9.5 and 5°C. Increasing the chlorine concentration from 1.0 to 5.0 mg/L gave T-99.99 values of 2.2, 2.4, and 33 minutes for HAV, poliovirus 1, and echovirus 1, respectively.

Table II, Appendix B also shows that pH also had a significant effect on virus inactivation efficiency. In both PBDFW and WC water experiments for poliovirus 1 and echovirus 1, increasing the pH from pH 4.5 to 9.5 greatly decreased virus inactivation efficiency. For example, the T-99.99 values for poliovirus 1 at 5°C in PBDFW with 1.0 mg/L free chlorine at pH 4.5 and 9.5 were 2.6 and 57 minutes, respectively. For echovirus under identical conditions the T-99.99 values were 7.0 and >>60 minutes at pH 4.5 and 9.5, respectively. These results indicate that poliovirus 1 and echovirus 1 are quite resistant to OCl^-, the form of free chlorine predominating at the higher pH. However, this pH effect was not observed for HAV under any of the conditions tested.

Temperature was also shown to have a significant effect on virus inactivation efficiency (Table II, Appendix B). The results of both PBDFW and WC water experiments show that increasing the temperature from 5°C to 25°C increased virus inactivation efficiency. For example, the T-99.99 values for HAV, poliovirus 1, and echovirus 1 were 7.6, 57, and >>60 minutes, respectively in PBDFW with 1.0 mg/L free chlorine at pH 9.5 and 5°C. Increasing the temperature to 25°C gave T-99.99 values of 2.0, 8.2 and 30 minutes for HAV, poliovirus 1, and echovirus 1, respectively.

Water quality was also shown to have a significant effect on virus disinfection efficiency by statistical analysis (Table II, Appendix B). However, the experimental data suggest that as long as chlorine demand was satisfied, virus inactivation rates were similar for both PBDFW and WC water experiments. It would be expected that in WC water, chlorine disinfection would be much less efficient than in HDF water due to chlorine demand. But, in fact, if the ranked data are examined (Table I, Appendix B), WC water was associated more often with detection limits of 1 minute or less. This phenomenon was due to the fact that higher concentrations of free chlorine were used in WC water experiments in order to meet the target free chlorine residuals of 1.0 and 5.0 mg/L at 30 minutes. Therefore, the variable of water quality was associated with chlorine concentration. From the ranked data of Appendix B Table I, it can be seen that the higher chlorine concentrations were usually associated with detection limits of 1 min or less.

E. Evaluation of Virus Inactivation Kinetics by the Watson Model

The results of experiments on halogen stability and virus inactivation in both PBDFW and WC water indicated that the halogen demand of the latter water, although greater than the former, still provided appreciable free chlorine residuals for virus inactivation and that viruses were rapidly and extensively inactivated in both test waters. Therefore, it was considered acceptable to pool the results of all virus inactivation experiments in both PBDFW and WC water to analyze virus inactivation data according to the Watson model. The Watson equation,

$$C^{n}T = K,$$

allows the analysis of the effect of changing disinfectant concentration on the rate of virus inactivation. The coefficient of dilution, n, is a measure of the order of the reaction (based on the van't Hoff model) and of the relative importance of disinfectant concentration on inactivation kinetics when compared to contact time. The values of n were computed for each virus at each pH level at 5°C by determining the slope of the fitted regression line when plotting log of chlorine concentration at

5°C versus log of the contact time to achieve 99% virus inactivation. For these calculations, it was assumed that once the initial chlorine demand of WC water was satisfied, there was no appreciable difference in virus inactivation kinetics between PBDFW and WC water. The estimted values of the coefficient of dilution, n, for each virus at each pH tested are summarized in Table 6. As noted previously, when the value of n is 1.0, disinfectant concentration and contact time are of equal importance and the disinfection kinetics can be described by Chick's Law as a simple first order reaction. When the value of n is >1, disinfectant concentration is more important than contact time, and when the value of n is <1, contact time is more important than disinfectant concentration in terms of influencing microbial inactivation kinetics. As shown in Table 6, the value of the coefficient of dilution, n, was close to 1.0 (between 0.9 and 1.0) in 3 of the 9 cases: HAV at pH 4.5 and 9.5 and echovirus 1 at pH 7.0. The coefficient of dilution was <0.9 in three cases: HAV at pH 7.0 (n = 0.22) and poliovirus 1 at pH 4.5 and 7.0 (n = 0.7 and 0.59, respectively). These results indicate that free chlorine concentration was somewhat less important than contact time for inactivation of HAV at pH 7.0 and poliovirus 1 at pH 4.5 and 7.0. For the remaining three cases the coefficient of dilution was >1.1: echo 1 at pH 4.5 (n = 1.58), and poliovirus 1 and echovirus 1 at pH 9.5 (n = 1.89 and 2.64, respectively). These results suggest that for echovirus 1 at pH 4.5 and 9.5 poliovirus 1 at pH 9.5, disinfectant concentration is more important than contact time for inactivation by free chlorine.

Overall, the range of values for the coefficient of dilution obtained in this study are not appreciably different from those previously reported for other enteroviruses (Haas and Karra, 1984; Hoff, 1986). For example, Sharp and Leong (1980) reported values of n of 0.69 and 3.23 for inactivation of poliovirus 1 (strain Brunhilde) at 20°C and pH 6.0 and 10.0, respectively. In this present study the values of n for poliovirus 1 show a similar pattern, with an n value <1 at low pH (n = 0.70 at pH 4.5) and a value of n >1 at high pH (n = 1.89 at pH 9.5)

F. Inactivation of Different Strains of HAV by Free Chlorine

Because the experiments described above were done only with the HM175 strain of HAV, it was of interest to determine if two other strains of HAV, MD-1 and CR326, would display comparable sensitivity to free chlorine. In these experiments echovirus 1 was included with HAV strains MD-1 and CR326 to act as an internal control and to verify that its inactivation kinetics were similar to those obtained in previous experiments. The results of experiments on inactivation of HAV strains MD-1 and CR326 by a 1 mg/l dose of free chlorine at pH 4.5 and 9.5 and 5°C are shown in Figure 18, where log N_t/N_o is plotted versus contact time and in Table 7 as T-99.99 values. The data of these

experiments are also given in Appendix Tables A25-27. These results show that the MD-1 strain of HAV was inactivated relatively rapidly (T-99.99 = 3.0 minutes at pH 4.5 and 4.1 minutes at pH 9.5) by a 1 mg/l dose of free chlorine and somewhat more rapidly than strain HM175 (T-99.99 = 5.8 minutes at pH 4.5 and 7.6 minutes at pH 9.5). The CR326 strain of HAV was inactivated at rates similar to HM175, with T-99.99 values of 3.2 minutes at pH 4.5 and 11 minutes at pH 9.5. As in previous experiments, echo 1 was inactivated relatively slowly by a 1 mg/l dose of free chlorine at pH 9.5 and 5°C, with a T-99.99 value of >>60 minutes (data not shown). The results of these experiments indicate that three strains of HAV, HM175, MD-1 and CR326, are relatively sensitive to free chlorine and that their inactivation rates are similar. For the three strains of HAV tested, the ranges of T-99.99 values are 3.0-5.8 minutes at pH 4.5 and 4.1-11 minutes at pH 9.5. The greater time for inactivation of all three strains of HAV at pH 9.5 and especially strain CR326, suggests that, like poliovirus 1 and ecovirus 1, inactivation rates are slower at the higher pH levels where OCl^- predominates.

G. Inactivation of HAV, Poliovirus 1 and Echovirus 1 by Iodine in Buffered HDFW

The mean results of duplicate experiments on inactivation of HAV, polio 1 and echo 1 by Army iodine (globaline tablets) at doses of 1 and 2 tablets per quart, pH 4.5, 7.0 and 9.5, and temperatures of 5 and 25°C are summarized in Tables 8 and 9, respectively, as times for 99.99% virus inactivation (T-99.99) and in Figures 19 to 30, where virus survival as log N_t/N_o is plotted versus contact time. The results are also presented in Appendix Tables A28-39. These results indicate that at pH 7.0 and 9.5, HAV is inactivated rapidly by 1 or 2 tablets of iodine per quart, with T-99.99 values of <2 minutes. At pH 4.5, the pH at which the iodine tablets are buffered, HAV inactivation rates are slower, with T-99.99 values ranging from 7.2 to 60 minutes, depending upon iodine dosage and temperature. As expected, HAV inactivation by iodine was greater at the higher temperature of 25°C than at 5°C. It should be noted that HAV inactivation rates were somewhat slower at the iodine dose of 2 tablets per quart than at the dose of 1 tablet per quart, at least at pH 4.5. The reasons for these somewhat aberrant results are uncertain. It is not due to failure to achieve target iodine concentration at pH 4.5 because 30- to 60-minute iodine residuals averaged 6.65 mg/l at a dose of 1 tablet per quart and 16.1 mg/l at a dose of 2 tablets per quart. Experiments at the two different iodine doses were often done using different virus preparations and different batches of iodinated water. Differences in the characteristics of the virus preparations, such as the size distribution of virus aggregates, or in the characteristics of the iodine solutions, such as the proportions of different iodine species in solution, could have contributed to differences in virus inactivation rates.

Results in Tables 8 and 9 and Figures 25 to 30 for inactivation of poliovirus 1 and echovirus 1 by 1 and 2 iodine tablets per quart (approximately 8 and 16 mg/l iodine dose, respectively) indicate that both viruses were inactivated relatively rapidly at pH 9.5 and temperatures of 5 and 25°C, with T-99.99 values of <10 minutes. At pH 4.5 and 5°C and 25°C, both poliovirus 1 and echovirus were inactivated relatively slowly, with T99.99 values ranging from <13 to 618 minutes for poliovirus and from <13 to 206 minutes for echovirus 1. Of the three viruses tested, poliovirus 1 was inactivated least rapidly under nearly all conditions tested. For all viruses there was a pattern of decreased inactivation at lower pH and lower temperature, with the slowest inactivation at the lower iodine dose and the lower temperature. The results of these experiments indicate that Army iodine did not achieve 99.99% inactivation of at least one of the three test viruses in PBDFW within 20 minutes, except at a dose of 2 tablets per quart and a temperature of 25°C. These findings have important implications for the current formulation and use of Army iodine because in clean, halogen demand free water, neither 1 nor 2 iodine tablets per quart will substantally inactivate some enteric viruses in a short time period at the acid pH levels the tablets are intended to achieve.

The mean results of replicate experiments on inactivation of HAV strain HM175, poliovirus 1 and echovirus 1 by an iodine dose of 1 or 2 tablets per quart in worst case water at pH 4.5, 7.0 and 9.5 and at 5°C are shown graphically in Figures 31 to 36 as log N_t/N_o and summarized in Table 10 as times for 99.99% virus inactivation. The data for individual experiments on iodine inactivation of test viruses in WC water are given in Appendix Tables A40-45. In worst case water no experiments were done at 25°C. In general the patterns of virus inactivation by iodine in worst case water are similar to those obtained in PBDFW. Of the three test viruses, HAV was inactivated most rapidly, with T-99.99 values of <2 minutes at iodine doses of 1 or 2 tablets per quart and pH levels of 7.0 and 9.5. However, at pH 4.5, HAV inactivation was relatively slow, with T-99.99 values of 140 and 104 minutes at iodine doses of 1 and 2 tablets per quart, respectively.

Both poliovirus 1 and echovirus 1 were inactivated more slowly than HAV, and overall, poliovirus 1 was inactivated least rapidly under the majority of test conditions. Both poliovirus 1 and echovirus 1 were inactivated most slowly at pH 4.5, with T-99.99 values ranging from 70 to 5,579 minutes. Inactivation of both poliovirus 1 and echovirus 1 was also relatively slow at pH 7.0. with T-99.99 values ranging from 19 to 366 minutes. Only at pH 9.5 were inactivation rates for poliovirus 1 and echovirus 1 relatively rapid, with T-99.99 values ranging from 1.2 to 64 minutes. Overall, virus inactivation rates of all test viruses were most rapid at the higher iodine dose of 2 tablets per quart and the highest pH (9.5), and they were least rapid at the lower iodine dose of 1 tablet per quart and the lowest pH (4.5).

Comparison of the data for virus inactivation by iodine in PBDFW and worst case water shows clearly that at corresponding conditions of iodine dose, temperature and pH, all test viruses were generally inactivated more slowly in worst case water than in PBDFW. These results suggest that the presence of organic acids and clay turbidity interfered with virus inactivation and reduced virus disinfection efficiency.

The finding that HAV is generally less resistant to iodine inactivation than are the other two test viruses is generally consistent with the results obtained using free chlorine. However, some differences can be noted between chlorine and iodine. At pH 9.5, all three viruses are inactivated most rapidly by iodine doses of 1 and 2 tablets per quart. In contrast, HAV is inactivated relatively rapidly at pH 9.5 by 1 and 5 mg/l free chlorine but poliovirus 1 and echovirus 1 are inactivated quite slowly, especially at 5°C. At pH 7.0, 1 and 5 mg/l free chlorine rapidly inactivated all three viruses (T99.99 <4 minutes), but iodine rapidly and consistently inactivated only HAV at pH 7.0. At pH 4.5, chlorine rapidly inactivates all 3 viruses (T-99.99 <8 minutes), but iodine inactivates them relatively slowly, especially at 5°C. These results demonstrate clear differences in the effectiveness of chlorine and iodine for virus inactivation at different pH levels, and they highlight the importance of maintaining optimum pH to achieve efficient virus inactivation.

H. Stability of Iodine in Mock Experimental Water

Because of concerns about the stability of iodine in test samples, mock virus inactivation experiments were done in which sucrose in PBDFW (mock virus stock) was added to iodine solutions containing 1 tablet of iodine per quart in PBDFW at pH 4.5, 7.0 and 9.5 and 5 and 25°C and in worst case water at pH 4.5, 7.0 and 9.5 and 5°C. These solutions were sampled at various times over 60 minutes and analyzed for residual iodine. As shown by the results on iodine stability in PBDFW in Figures 37 and 38, iodine at a dose of 1 tablet per quart was quite stable in PBHDFW at 5 and 25°C at both pH 4.5 and 7.0, with iodine residial losses of less than 10% of initial concentrations in 60 minutes. At pH 9.5, however, iodine losses were considerably greater. At 5°C iodine residual decreased over 60 minutes from about 8 mg/l to about 6 mg/l, a decrease of 25% from the initial concentration. At 25°C, iodine residual decreased over 60 minutes from about 7.5 mg/l to about 3 mg/l, a decrease of at least 4.5 mg/l, representing a loss of at least 60% from the initial concentration.

As shown in Tables 11-16 and Figure 39, iodine losses in mock inactivation experiments in worst case water at 5°C were greater than those in PBDFW. Iodine losses over 60 minutes as a percentage of initial concentrations ranged from <5 to >95% at a dose of 1 tablet per quart and fromt <5 to 87% at a dose of 2

tablets per quart. Losses were least (<32%) at pH 4.5, somewhat higher (<75%) at pH 7.0 and highest at pH 9.5.

The results of these iodine stability experiments are generally consistent with those of actual virus inactivation experiments, but in virus inactivation experiments, iodine losses at pH 9.5 were much greater than they were in mock inactivation studies. Iodine residuals in actual test samples of virus inactivation experiments are summarized in Figure 40. At pH 4.5 and 7.0, iodine losses over periods of 30 or 60 minutes were <2 mg/l at a dose of 1 tablet per quart and <4 mg/l at a dose of 2 tablets per quart. Iodine losses were somewhat greater at 25°C than at 5°C. At pH 9.5, iodine losses were so great that at the lower iodine dose of 1 tablet per quart, there was no detectable iodine residual remaining at 60 minutes. At a dose of 2 tablets per quart and pH 9.5, iodine loss at 30 minutes was about 87% of the initial concentration of 16.6 mg/l. These results indicate that iodine is relatively unstable at pH 9.5, as has been previously shown (Chang, 1958). At pH 9.0 and higher, hypoiodous acid, HOI, undergoes dissociation to form hypoiodite ion, IO^-, which quickly decomposes to iodate and iodide.

I. Inactivation of Different Strains of HAV by Iodine

Three strains of HAV, HM175, MD-1 and CR326, were compared for their inactivation by a dose of 1 tablet of iodine per quart in PBDFW at 5°C. This test condition was selected because in other experiments using PBDFW it gave the greatest survival of HAV and other test viruses. The results of these experiments are shown in Figure 41, where log N_t/N_o is plotted versus contact time, and in Table 17 as T-99.99 values. The T-99.99 values for HAV strains HM175, MD-1 and CR-326 were 43, 66 and 33 minutes, respectively. Thus, all three strains of HAV inactivated at similar rates, with no more than a two-fold difference in T-99.99 values between any two strains. The T-99.99 value for HM175 obtained in these experiments was quite similar to that obtained in previous experiments done under the same conditions.

J. Single Particle Approximation Analyses

Tables 18-20 summarize the results of the single particle approximation (SPA) experiments. Due to a limited amount of virus an SPA was run at 20°C and at pH 4.5, 7.0 and 9.5 on HAV sucrose gradient fractions in PBDFW only. Both PBDFW and WC SPA were run on poliovirus 1 and echovirus 1 sucrose gradient fractions. The results are presented as the percent of the total virus fraction that represents single virus particles. In both PBDFW and WC water, the percent of singles in the singles virus PBS controls were above 100%. This may have been due to the breaking up of virus that may have been aggregated or to sources of calculation error in estimating single particle content. Therefore, the singles virus PBS controls for each virus in either PBDFW or WC waters were averaged and normalized to 100%.

The percent singles of each test sample were then normalized to their corresponding singles virus PBS control.

The SPA data for HAV in PBDFW water at 20°C at pH 4.5, reveal that all fractions (singles and small, medium, and large aggregates) are highly aggregated with estimated percent singles of 3.5 to 19.2 percent (Table 18). At pH 7.0 and 9.5, the singles and small aggregate fractions are moderately aggregated, and the medium and large aggregate fractions are highly aggregated. An exception to this is the medium aggregate fraction at pH 9.5, which appears to be monodisperse.

Results of the SPA experiments for poliovirus 1 and echovirus 1 in PBDFW at pH 4.5 are very similar to those for HAV (Tables 19-20). All fractions at pH 4.5 were aggregated, although the degree of aggregation is not as great as was observed for HAV. However, in WC water the same poliovirus 1 and echovirus 1 fractions at pH 4.5 were highly dispersed. At pH 7.0 and 9.5 in both HDF and WC water, all poliovirus 1 and echovirus 1 fractions, with two exceptions, were shown to be monodispersed. Therefore, it appears from the SPA data that the poliovirus 1 and echovirus 1 sucrose gradient fractions were not aggregated at pH 7.0 and 9.5 in PBDFW water and at all pH levels in WC water and that the gradient profiles for these two viruses were not the same as for HAV.

Virus control data from the free chlorine inactivation experiments appear to show similar aggregation phenomena as seen in SPA experiments at pH 4.5 in HDF water (Appendix A). For some WC water experiments PBDFW virus controls (virus in buffered demand free water) and WC virus controls (virus in buffered WC water) were run simultaneously. At pH 4.5, all 30 minute PBDFW virus control titers were consistently lower than 30 minute WC virus control titers. Large differences in virus titers can be seen between PBDFW and WC water 30 minute virus controls for each virus at each pH. At pH 4.5 for poliovirus 1 and echovirus 1, the 30 minute virus control titers for WC water are up to 9 times higher than those for PBDFW water. For HAV, at pH 4.5 the 30 minute virus control titers for WC water are up to 25 times higher than PBDFW. At pH 7.0 and 9.5 for all viruses there is not much difference between the 30 minute virus control titers in PBDFW or WC water. The drop in virus titer at pH 4.5 in PBDFW may be due to the increased aggregation of virus at this lower pH.

In addition, the decrease in virus titer at pH 4.5 in PBDFW can be seen over time by examining the starting (0 minute) and ending (30 minute) virus control titers. An examination of the 0 and 30 minute virus control titers in PBDFW water at pH 4.5 reveals that there was up to a 6 fold decrease in virus titers over the 30 minute experiment. For the same virus controls in WC water only up to a 1.6 fold decrease was noted. For all viruses at pH 7.0 and 9.5 in both PBDFW and WC waters, the 0 minute virus control

titers did not differ much from the 30 minute virus control titers.

V. SUMMARY AND CONCLUSIONS

Chlorine Disinfection.

The general order of chlorine effectiveness on viruses (from most effected to least effected virus) was: HAV > poliovirus 1 > echovirus 1. Hepatitis A virus was generally more sensitive to free chlorine than either poliovirus 1 or echovirus 1. In particular, HAV was considerably more sensitive to free chlorine at pH 9.5 than either poliovirus 1 and echovirus 1. The finding that HAV was relatively sensitive to free chlorine is generally consistent with the reported findings of Grabow et al. (1983) and Sobsey et al. (1988b) and in contrast to the findings of Peterson et al. (1983). The efficiency of virus inactivation by free chlorine was significantly influenced by pH, temperature, water quality and chlorine concentration. HAV strains HM175, MD-1 and CR326 were shown to be similarly sensitive to free chlorine at all conditions tested, and it was possible to achieve 99.99% inactivation of all three strains within 11 minutes at a free chlorine dose of 1 mg/l and a temperature of 5°C. For poliovirus 1, it was possible to achieve 99.99% inactivation under all conditions tested within 30 minutes, except for a 1 mg/l dose of free chlorine at pH 9.5 and 5°C. For echovirus 1, 99.99% virus inactivation was achieved within 30 minutes, except for the following conditions: 1.0 and 5.0 mg/l free chlorine at pH 9.5 and 5°C in PBDFW, and 3 mg/l free chlorine at pH 9.5 and 5°C in WC water. Under most conditions tested, inactivation of HAV and poliovirus was so rapid that it was difficult to adequately describe the rate of reaction or the reaction kinetics.

Both poliovirus 1 and echovirus 1 demonstrated increased resistance to free chlorine at increasing pH. This is in general agreement with the results of other investigators, who have shown that enteroviruses are more resistant to OCl^-, the predominant free chlorine species at high pH, than HOCl, the predominant free chlorine species at lower pH. Inactivation times of HAV, poliovirus 1 and echovirus 1 at pH 4.5 by free chlorine in both PBDFW and WC water were similar to or slightly slower than inactivation times at pH 7.0. This is somewhat unusual since the proportion of HOCl is greater at pH 4.5 than at 7.0. These findings may be related to an increased degree of virus aggregation at pH 4.5 or to pH-dependent changes in the conformational forms of the viruses. However, the results of worst case water SPA experiments for poliovirus 1 and echovirus 1 suggest that viral aggregation was probably not responsible for the results obtained, because at pH 4.5, neither virus was aggregated. The role of clay particles in worst case water on virus inactivation at the different pH levels is uncertain. It is possible that there were interactive forces between clay perticles and viruses (both aggregated and dispersed) and that these interactions also changed with pH. However, no such changes could were observed in SPA experiments and the data for virus inactivation experiments do not suggest such effects.

Although not specifically investigated in this study, previous investigations have shown that conformational shifts in the virus capsid arrangement may influence the rate and extent of virus inactivation (Young and Sharp, 1985).

The coefficient of dilution, n, the exponent value of the disinfectant concentration term in the Watson equation, varied for both the type of virus and the pH of the reaction when using free chlorine as the disinfectant. For inactivation of HAV, chlorine concentration was about as important as contact time at pH 4.5 and 9.5 ($n = 1$) but at pH 7.0, chlorine concentration was apparently less important than contact time ($n < 1$). For inactivation of poliovirus 1, chlorine concentration was less important than contact time at pH 4.5 and 7.0 ($n < 1$), but at pH 9.5, chlorine concentration was more important than contact time ($n > 1$). For inactivation of echovirus 1, chlorine concentration was more important than contact time at pH 4.5 and 9.5 ($n > 1$), but at pH 7.0, chlorine concentration and contact time were of equal importance ($n = 1$).

Overall, it appears that both poliovirus 1 and echovirus 1 are reasonably useful viruses as conservative predictors of HAV inactivation by free chlorine. This is because under nearly all conditions tested, poliovirus 1 and echovirus 1 were somewhat more difficult to inactivate with chlorine than was HAV. However, the results of these studies indicate that echovirus 1 and poliovirus 1 are not accurate models for the response of HAV to free chlorine. HAV is generally more sensitive to free chlorine than either poliovirus 1 or echovirus 1. HAV strain HM175 is probably an adequate model to predict the reponses of other strains of HAV to free chlorine, because HM175 and the other two strains of HAV tested (MD-1 and CR326) showed similar inactivation kinetics by free chlorine.

The results of experiments on virus inactivation in worst case water suggest that when enough free chlorine is used to satisfy the chlorine demand and achieve a target free chlorine residual, then it is possible to obtain >99.99% virus inactivation in time periods comparable to those achieved in chlorine demand free water. Thus, as long as target free chlorine residuals are achieved, the presence of interfering turbidity (bentonite clay) and organic matter (humic and fulvic acids) does not appreciably interfere with virus inactivation, regardless of the pH and temperature of the water.

Single particle approximation tests done in PBDFW suggested that all three test viruses were aggregated at pH 4.5, and HAV was the most heavily aggregated. These findings are in general agreement with those of other investigators, and may explain the apparent lack of increased susceptibility of test viruses to free chlorine at pH 4.5 compared to pH 7.0. At pH 7.0 and 9.5 in PBDFW, both poliovirus 1 and echovirus 1 were shown by SPA analysis to be almost completely dispersed. HAV was somewhat aggregated at pH

7.0, but at pH 9.5, it was monodispersed. These results suggest that attempts to create stable preparations of virus aggregates are not always successful. SPA studies done in WC water indicated that poliovirus 1 and echovirus 1 were monodispersed at all pH levels tested. These results at pH 4.5 may be due to virus-organic acid interactions that prevent the formation of virus aggregates. The results of WC water SPA studies suggest that there was little if any virus-clay interactions, probably because organic acids interfered with virus adsorption to clay particle surfaces.

An important conclusion that can be drawn from the results of virus inactivation experiments using free chlorine is that all three test viruses are inactivated extensively (>99.99%) by a 5 mg/l dose of free chlorine in about 30 minutes or less. These results suggest that the Army disinfection criterion of a 5 mg/l free chlorine residual after 30 minutes for Army field water purification units (reverse osmosis) is sufficient to achieve substantial (>99.99%) inactivation of HAV and other enteroviruses.

B. Iodine Disinfection

The results of this study demonstrate that HAV is generally inactivated more rapidly and extensively by iodine than are poliovirus 1 and echovirus 1. At iodine doses of 1 and 2 tablets per quart in in PBDF water, HAV strain HM175 was inactivated by 99.99% or 4 $\log_{10}$ in 60 minutes or less at all test conditions. Two other strains of HAV, CR326 and MD-1, were inactivated by 99.99% in time periods similar to that for HM175 at comparable test conditions. In contrast, the same iodine doses did not give 99.99% inactivation of polio 1 and echo 1 at some test conditions. These conditions were 1 tablet per quart doses of iodine at pH 4.5 and 7.0 and 5°C and 2 tablets per quart doses of iodine at pH 4.5 and 5°C. Overall, the order of virus inactivation by iodine was: HAV > echo 1 > polio 1.

In PBDF water containing 10 mg/l of organic acids and 5 NTU of bentonite clay turbidity at 5°C, virus inactivation by the same doses of iodine at comparable conditions of pH and temperature was less efficient than in PBDF free water only. Estimated times for 99.99% virus inactivation were >60 minutes for all test viruses at pH 4.5 and for polio 1 at pH 7.0. The reduced efficiency of virus inactivation in water containing organic matter and turbidity was not unexpected, as previous studies have documented that both dissolved organic matter and particulates (turbidity) interfere with the inactivation of viruses and other microbes by strongly oxidizing chemical disinfectants (Sobsey, 1989).

Virus inactivation by iodine was generally more rapid and extensive at a dose of 2 tablets per quart than at a dose of 1 tablet per quart. This finding was to be expected because

typically, viruses and other microbes are inactivated more efficiently at greater disinfectant dose (Sobsey, 1989). In PBDF water virus inactivation by iodine was found to be more rapid and extensive at 25°C than at 5°C. These results support those of other studies in which inactivation of viruses and other microbes by chemical disinfectants is more efficient at higher temperature (Sobsey, 1989).

Virus inactivation by iodine was generally greatest at pH 9.5, less at pH 7.0 and least at pH 4.5. The more rapid inactivation of viruses by iodine at alkaline pH is consistent with the findings of previous studies. For example, Cramer at al. (1976) reported that polio 3 and coliphage f2 in wastewater were inactivated more rapidly and extensively by iodine at pH 10 than at pH 4. More recently, Alvarez and O'Brien (1982) found that a 2.5 mg/l dose of iodine in buffered, demand free water at 25°C inactivated polio 1 more rapidly at pH 10 than at pH 6. The greater inactivation of viruses by iodine at alkaline pH is probably related to the fact that at alkaline pH levels, I_2 hydrolyzes to form HOI, and HOI is a more potent virucide than I_2. The more efficient inactivation of viruses by iodine at higher pH poses a serious dilemma for the use of iodine as a water disinfectant. In contrast to viruses, enteric bacteria and cysts of Entamoeba histolytica are inactivated more efficiently by iodine at lower pH where iodine exists primarily as I_2 (Chang, 1958). Thus, iodine has a different pH optimum for virus inactivation than for bacteria and protozoan cyst inactivation. The globaline tablets used in this present study are normally buffered to low pH in order to optimize the inactivation of bacteria and protozoan cysts (Chang, 1958).

Overall, the results of this present study suggest that iodine at doses of 2 tablets per quart is not always an efficient virucide in water. In both PBDF water and PBDF water containing 10 mg/l organic acids and 5 NTU of clay turbidity, 99.99% or 4 $\log_{10}$ inactivation of some test viruses was not achieved rapidly at some test conditions. Specifically, virus inactivation was relatively inefficient at the lower iodine dose of 1 tablet per quart, at the lower temperature of 5°C, at neutral and acid pH, and in water containing nominal levels of dissolved organic matter and clay turbidity. These findings suggest that caution must be exercised in using iodine for disinfection of personal and other water supplies because rapid and extensive virus inactivation will not be achieved under certain water quality conditions.

C. EFfects of Aggregation on Virus Inactivation.

The effects of pH and water quality on virus aggregation in PBDFW indicate that it is difficult to control the state of virus aggregation, even when virus aggregates of defined size are carefully prepared and selected for seeding of test waters. Therefore, it may be necessary to develop alternative models for

aggregated or particle-associated viruses. Recently, this laboratory reported on the comparative inactivation of dispersed and cell-associated hepatitis A virus by free chlorine and monochloramine in PBDFW (Sobsey et al., 1991). Cell-associated HAV, which can be taken as a model for clumped or solids-protected viruses in water, were inactivated more slowly and less extensively than dispersed HAV by free chlorine and monochloramine. Perhaps cell-associated viruses are better models for clumped or solids-protected viruses in fecally contaminated natural waters than are the highly purified but aggregated viruses used in this present study. Future studies should be done to investigate various models for solids-protected or solids-associated viruses in water and their inactivation by drinking water disinfectants.

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Table 1. Time for 99.99% Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine in PBDFW at pH 4.5, 7.0 and 9.5; 5°C and 25°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
		5°C	
HAV	5.2	0.7	7.3
Polio 1	4.2	1.2	59
Echo 1	7.1	3.6	>>60
		25°C	
HAV	2.6	<0.33**	2.2
Polio 1	0.8	0.4	8.4
Echo 1	0.8	1.4	32

*Times for 99.99% inactivation estimated by linear regression analysis of $\log_{10}$ virus survival versus time; mean results of duplicate experiments for each condition.

**Less than (<) values are based on virus detection limits in samples assayed; where one replicate trial gave a measurable virus titer and the other replicate trial was indeterminate, the indeterminate value was averaged with the detection limit.

Table 2. Time for 99.99% Inactivation of HAV, Polio 1 and Echo 1 by 5 mg/l Free Chlorine in PBDFW at pH 4.5, 7.0 and 9.5; 5°C and 25°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
		5°C	
HAV	2.3	1.0	2.7
Polio 1	0.5	1.1	5.1
Echo 1	0.4	0.6	34
		25°C	
HAV	1.1	0.5	1.0
Polio 1	0.4	0.2	4.4
Echo 1	0.5	0.4	11

*Times for 99.99% inactivation estimated by linear regression analysis of $\log_{10}$ virus survival versus time; mean results of duplicate experiments for each condition.

**Less than (<) values are based on virus detection limits in samples assayed; where one replicate trial gave a measurable virus titer and the other replicate trial was indeterminate, the indeterminate value was averaged with the detection limit.

Table 3. Time for 99.99% Inactivation of HAV (HM175), Polio 1 and Echo 1 by a 3 mg/l Dose of Free Chlorine in Worst Case Water at pH 4.5, 7.0 and 9.5; 5°C and 25°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
		5°C	
HAV	0.6	0.5	0.5
Polio 1	1.2	0.6	4.6
Echo 1	3.4	1.7	72
		25°C**	
HAV	0.5	<0.4	0.4
Polio 1	0.6	<0.4	1.2
Echo 1	<0.4	<0.5	6.6

*Times for 99.99% inactivation estimated by linear regression analysis of $\log_{10}$ virus survival versus time;
**mean results of duplicate experiments for each condition.
Less than (<) values are based on virus detection limits in samples assayed; where one replicate trial gave a measurable virus titer and the other replicate trial was indeterminate, the indeterminate value was averaged with the detection limit.

Table 4. Time for 99.99% Inactivation of HAV (HM175), Polio 1 and Echo 1 by a 7 mg/l Dose of Free Chlorine in Worst Case Water at pH 4.5, 7.0 and 9.5; 5°C and 25°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
		5°C	
HAV	0.6	1.2	0.6
Polio 1	0.7	0.4	1.5
Echo 1	<0.4	0.5	4.2
		25°C	
HAV	<0.4	<0.4	<0.4
Polio 1	<0.4	<0.4	<0.4
Echo 1	<0.4	<0.3	<0.4

*Times for 99.99% inactivation estimated by linear regression analysis of $\log_{10}$ virus survival versus time;
**mean results of duplicate experiments for each condition.
Less than (<) values are based on virus detection limits in samples assayed; where one replicate trial gave a measurable virus titer and the other replicate trial was indeterminate, the indeterminate value was averaged with the detection limit.

Table 5. Stability of Free Chlorine in PBDF and Worst Case Waters Dosed with a Mock Virus Mixture at 5°C.[a]

Water	pH	Free Chlorine Residual (mg/l) at: 0 min.	5 Min.	30 Min.
PBDF	4.5	3.25	3.25	3.22
	7.0	3.26	3.26	3.2
	9.5	3.25	3.25	3.21
Worst Case	4.5	3.25	3.18	2.68
	7.0	3.26	2.51	2.04
	9.5	3.25	2.70	2.20

[a]PBDF water is 0.01M phosphate buffer, demand free; mock virus mixture was 23% sucrose diluted 1:10 in PBDF.

Table 6. Values for n for Inactivation of HAV Poliovirus 1, and Echovirus 1 by Free Chlorine.[a]

Virus	pH 4.5		pH 7.0		pH 9.5	
	n	r^{2b}	n	r^2	n	r^2
HAV	1.07	0.86	0.22	0.53	1.10	0.72
Polio 1	0.70	0.93	0.59	0.94	1.89	0.99
Echo 1	1.58	0.92	1.07	0.97	2.64	0.98

[a] n_2 is the exponent of C in the Watson equation ($C^n t = K$).
[b] r^2 is the correlation coefficient.

Table 7. Time for 99.99% Inactivation of HAV Strains HM175, MD-1 and CR326 by 1 mg/l Free Chlorine in PBDFW at pH 4.5 and 9.5 and 5°C

HAV Strain	Time (Min.) for 99.99% Inactivation pH 4.5	pH 9.5
HM175	5.8	7.6
MD-1	3.0	4.1
CR326	3.2	11.0

Table 8. Time for 99.99% Inactivation of HAV (HM175), Polio 1 and Echo 1 by 1 Tablet Per Quart of Iodine in PBDF Water at pH 4.5, 7.0 and 9.5 and 5°C and 25°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
		5°C	
HAV	37	1.1	0.4
Polio 1	618	132	<1.5
Echo 1	206	63	<4.2
		25°C	
HAV	7.2	<0.4	<0.3
Polio 1	54	<3.9	0.7
Echo 1	<13	<4.9	<1.7

*Times for 99.99% inactivation estimated by linear regression analysis of $\log_{10}$ virus survival versus time; mean results of duplicate experiments for each condition.
**Less than (<) values are based on virus detection limits in samples assayed; where one replicate trial gave a measurable virus titer and the other replicate trial was indeterminate, the indeterminate value was averaged with the detection limit.

Table 9. Time for 99.99% Inactivation of HAV (HM175), Polio 1 and Echo 1 by 2 Tablets Per Quart of Iodine in PBDF Water at pH 4.5, 7.0 and 9.5 and 5°C and 25°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
		5°C	
HAV	60	<1.1**	<0.3
Polio 1	162	<79	9.8
Echo 1	63	<12	2.2
		25°C	
HAV	7.8	<0.4	<0.4
Polio 1	<13	4.0	<0.5
Echo 1	7.0	<1.2	0.6

*Times for 99.99% inactivation estimated by linear regression analysis of $\log_{10}$ virus survival versus time; mean results of duplicate experiments for each condition.
**Less than (<) values are based on virus detection limits in samples assayed; where one replicate trial gave a measurable virus titer and the other replicate trial was indeterminate, the indeterminate value was averaged with the detection limit.

Table 10. Time for 99.99% Inactivation of HAV (HM175), Polio 1 and Echo 1 by 1 and 2 Tablets Per Quart of Iodine in Worst Case Water at pH 4.5, 7.0 and 9.5 and 5°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
	1 Tablet Per Quart		
HAV	140	1.8	<0.4
Polio 1	1,000	366	2.2
Echo 1	5,570	49	3.7
	2 Tablets Per Quart		
HAV	104	0.8	<0.4
Polio 1	353	82	64
Echo 1	70	19	1.2

*Times for 99.99% inactivation estimated by linear regression analysis of $\log_{10}$ virus survival versus time; mean results of duplicate experiments for each condition.

**Less than (<) values are based on virus detection limits in samples assayed; where one replicate trial gave a measurable virus titer and the other replicate trial was indeterminate, the indeterminate value was averaged with the detection limit.

Table 11. Amount and Percentage of 2 Tablets/Quart Iodine Remaining in PBDF Control, Worst Case Control and Worst Case Test Waters at pH 4.5 and 5°C

Time Min.	PBDF Control Mg/L	Percent	Worst Case Control Mg/I	Percent	Test Sample Mg/L	Percent
0	16.4	100	----	----	----	----
0.5	----	---	15.2	93.1	----	----
30	16.1	98.4	14.9	91.0	14.1	86.2
60	15.8	96.4	14.4	87.7	13.4	81.5

Table 12. Amount and Percentage of 2 Tablets/Quart Iodine Remaining in PBDF Control, Worst Case Control and Worst Case Test Waters at pH 7.0 and 5°C

Tim Min.	PBDF Control Mg/L	Percent	Worst Case Control Mg/L	Percent	Test Sample Mg/L	Percent
0	16.5	100	----	----	----	----
	----	---	14.1	85.5	----	----
	16.1	97.8	12.5	75.8	11.5	69.8
	15.6	94.5	11.8	71.6	10.6	64.5

Table 13. Amount and Percentage of 2 Tablets/Quart Iodine Remaining in PBDF Control, Worst Case Control and Worst Case Test Waters at pH 9.5 and 5°C

Time	PBDF Control		Worst Case Control		Test Sample	
Min.	Mg/L	Percent	Mg/L	Percent	Mg/L	Percent
0	16.6	100	----	----	----	----
0.5	----	---	13.5	81.3	----	----
15	14.1	84.7	10.6	63.9	----	----
30	12.5	75.2	9.1	54.7	2.2	13.0

Table 14. Amount and Percentage of 1 Tablet/Quart Iodine Remaining in PBDF Control, Worst Case Control and Worst Case Test Waters at pH 4.5 and 5°C

Time	PBDF Control		Worst Case Control		Test Sample	
Min.	Mg/L	Percent	Mg/L	Percent	Mg/L	Percent
0	8.6	100	----	----	----	----
0.5	----	---	7.1	82.9	----	----
30	8.3	96.9	6.6	76.5	6.2	71.6
60	7.9	91.6	6.3	73.7	5.8	67.2

Table 15. Amount and Percentage of 1 Tablet/Quart Iodine Remaining in PBDF Control, Worst Case Control and Worst Case Test Waters at pH 7.0 and 5°C

Time	PBDF Control		Worst Case Control		Test Sample	
Min.	Mg/L	Percent	Mg/L	Percent	Mg/L	Percent
0	8.8	100	----	----	----	----
0.5	----	---	7.1	81.1	----	----
30	8.6	98.5	5.9	67.0	5.5	62.9
60	8.4	95.6	5.4	61.9	4.9	55.5

Table 16. Amount and Percentage of 1 Tablet/Quart Iodine Remaining in PBDF Control, Worst Case Control and Worst Case Test Waters at pH 9.5 and 5°C

Time Min.	PBDF Control Mg/L	PBDF Control Percent	Worst Case Control Mg/L	Worst Case Control Percent	Test Sample Mg/L	Test Sample Percent
0	9.3	100	----	----	----	----
0.5	----	---	5.7	61.1	----	----
30	7.8	83.9	3.3	35.4	<0.5	<5.4
60	6.3	68.1	2.5	26.5	<0.5	<5.4

Table 17. Time for 99.99% Inactivation of HAV Strains HM175, MD-1 and CR326 by 1 Tablet of Iodine per Quart in PBDF Water at pH 4.5 and 5°C

HAV Strain	Time (Minutes) for 99.99% Inactivation
HM175	43
MD-1	66
CR326	33

Table 18. Single Particle Approximation of HAV in Sucrose Gradient Fractions in PBDF Water at 20°C

Fraction Type[a]	Percent Single Particles[b] pH 4.5	pH 7.0	pH 9.5
Singles	7.3	69.0	81.9
Small	3.5	49.5	75.0
Medium	19.2	8.5	227.0
Large	14.5	22.9	30.3

[a]Sucrose gradient fractions were arbitrarily divided into four size classes desinated singles and small, medium and large aggregates based on sucrose gradient profiles.
[b]Percentage of each sucrose gradient fraction that were single particles, normalized to a PBS virus control sample.

Table 19. Single Particle Approximation of Poliovirus 1 in Sucrose Gradient Fractions in PBDF and Worst Case Waters Water at 20°C

Fraction Type[a]	Percent Single Particles[b] pH 4.5	pH 7.0	pH 9.5
	PBDF (Phosphate Buffered Demand Free) Water		
Singles	55.8	79.0	145.6
Small	41.9	112.4	126.2
Medium	42.6	118.4	52.9
Large	37.3	113.0	115.9
	Worst Case Water		
Singles	76.4	143.7	76.4
Small	110.3	210.2	106.2
Medium	166.0	166.4	143.4
Large	82.5	81.8	70.3

[a]Sucrose gradient fractions were arbitrarily divided into four size classes desinated singles and small, medium and large aggregates based on sucrose gradient profiles.
[b]Percentage of each sucrose gradient fraction that were single particles, normalized to a PBS virus control sample.

Table 20. Single Particle Approximation of Echovirus 1 in Sucrose Gradient Fractions in PBDF and Worst Case Waters Water at 20°C

Fraction Type[a]	Percent Single Particles[b] pH 4.5	pH 7.0	pH 9.5
	PBDF (Phosphate Buffered Demand Free) Water		
Singles	50.7	165.0	120.6
Small	51.9	160.0	130.3
Medium	50.7	197.7	177.1
Large	76.5	203.9	133.0
	Worst Case Water		
Singles	94.0	110.9	84.5
Small	121.1	140.3	244.0
Medium	86.1	88.6	85.0
Large	124.2	61.0	110.7

[a]Sucrose gradient fractions were arbitrarily divided into four size classes desinated singles and small, medium and large aggregates based on sucrose gradient profiles.
[b]Percentage of each sucrose gradient fraction that were single particles, normalized to a PBS virus control sample.

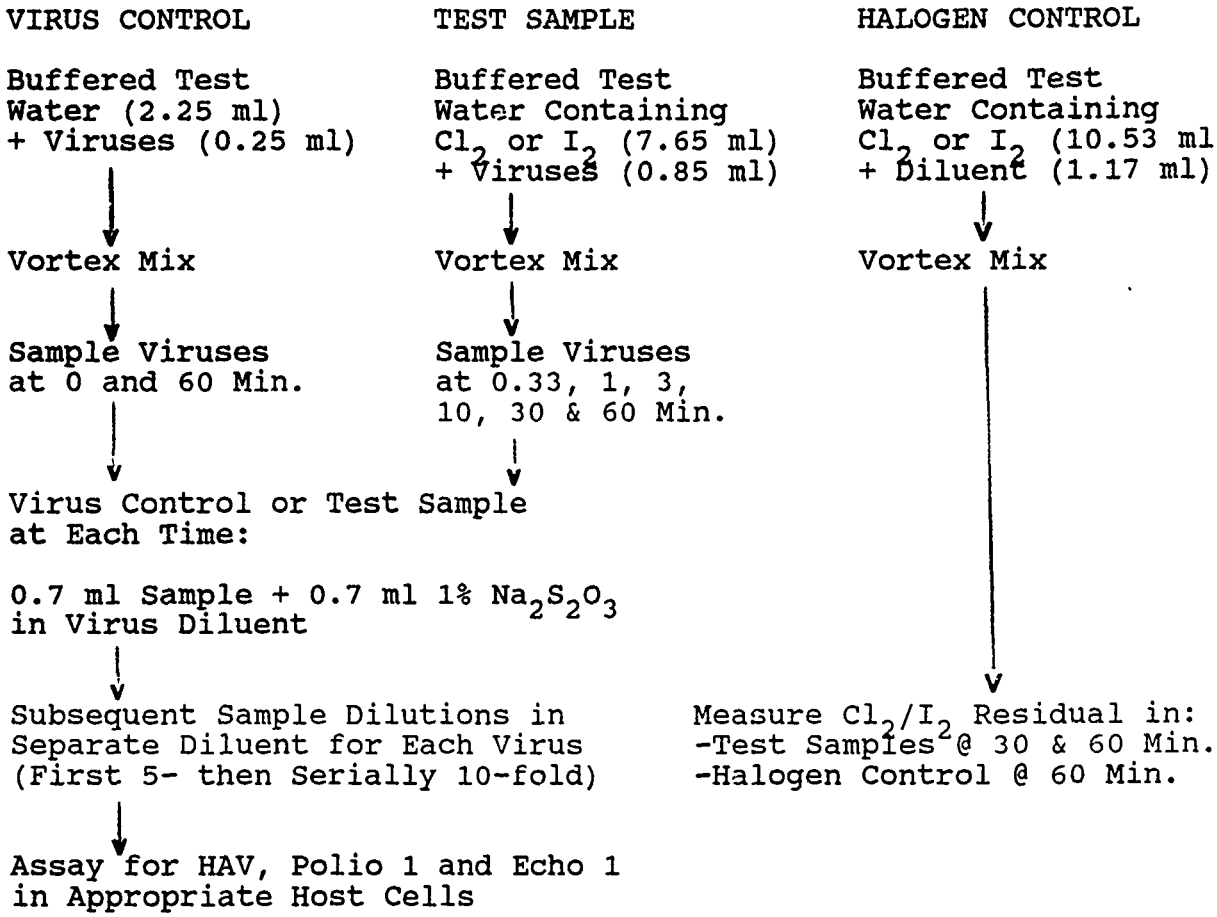


Figure 1. FLOW DIAGRAM OF PROTOCOL FOR HALOGEN DISINFECTION EXPERIMENTS

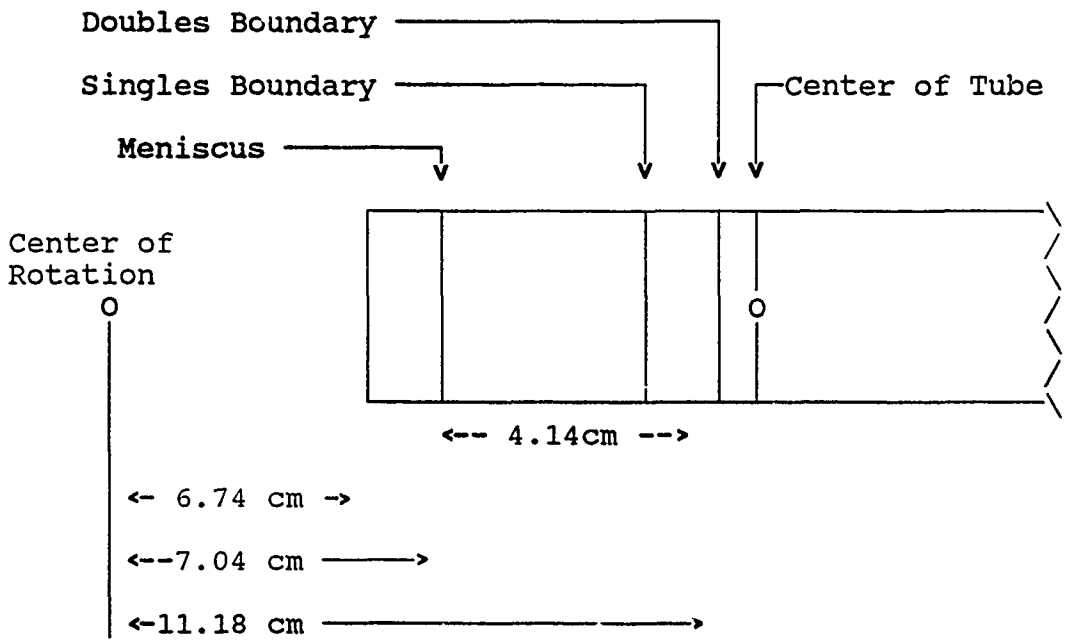


Figure 2. Measurements used in the SPA test. Adapted from Floyd and Sharp. 1977.

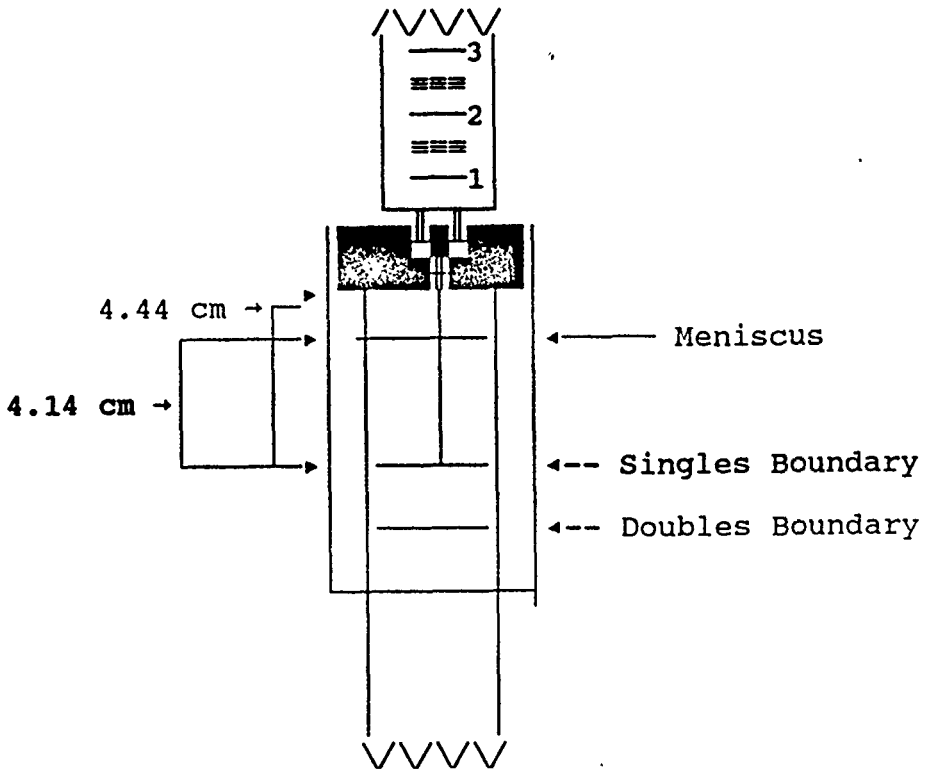


Figure 3. Apparatus for collecting liquid from the top one-half of the ultracentrifuge tube.

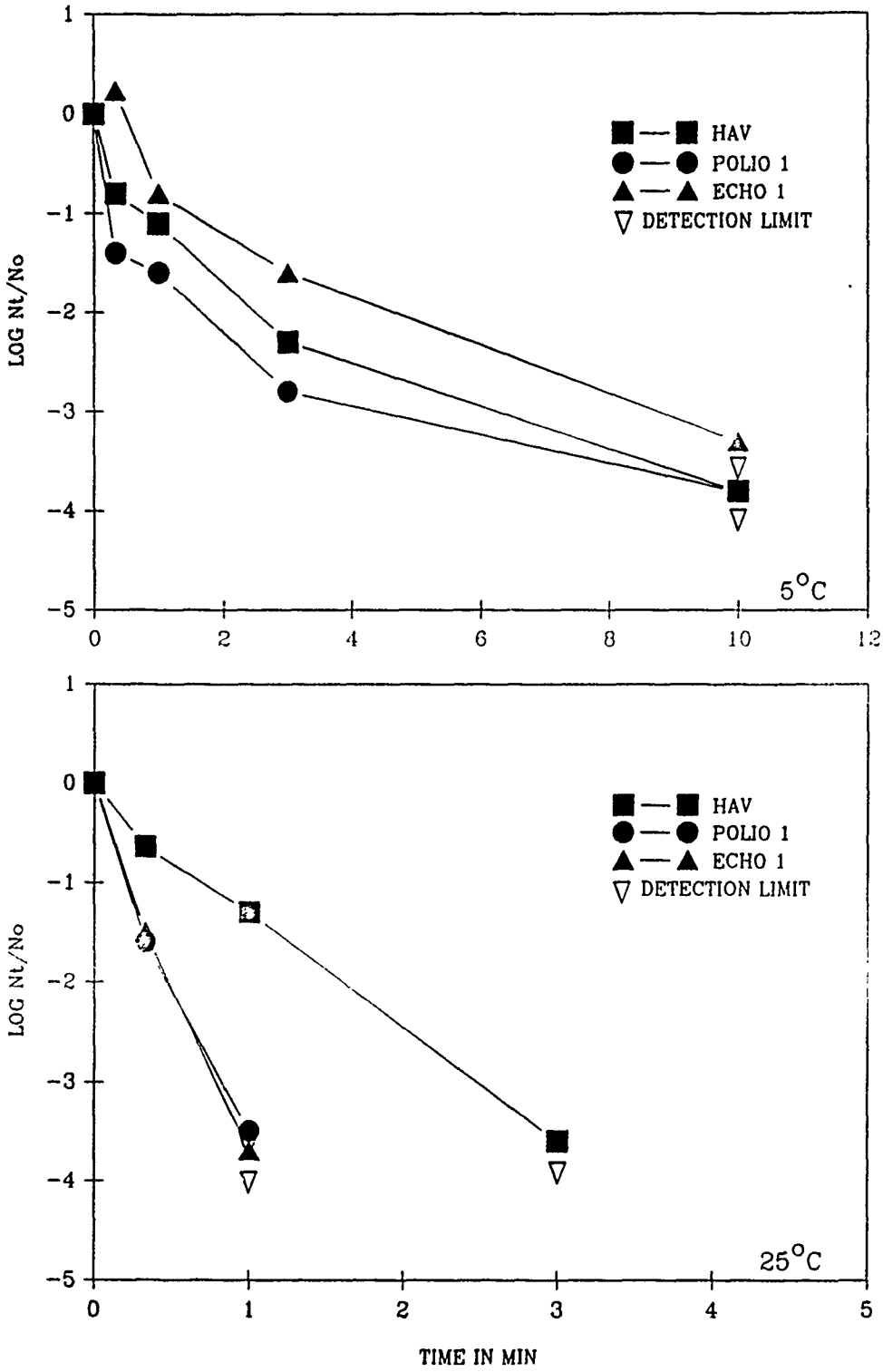


Figure 4. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 1.0 mg/L at pH 4.5, 5°C and 25°C in halogen demand free water

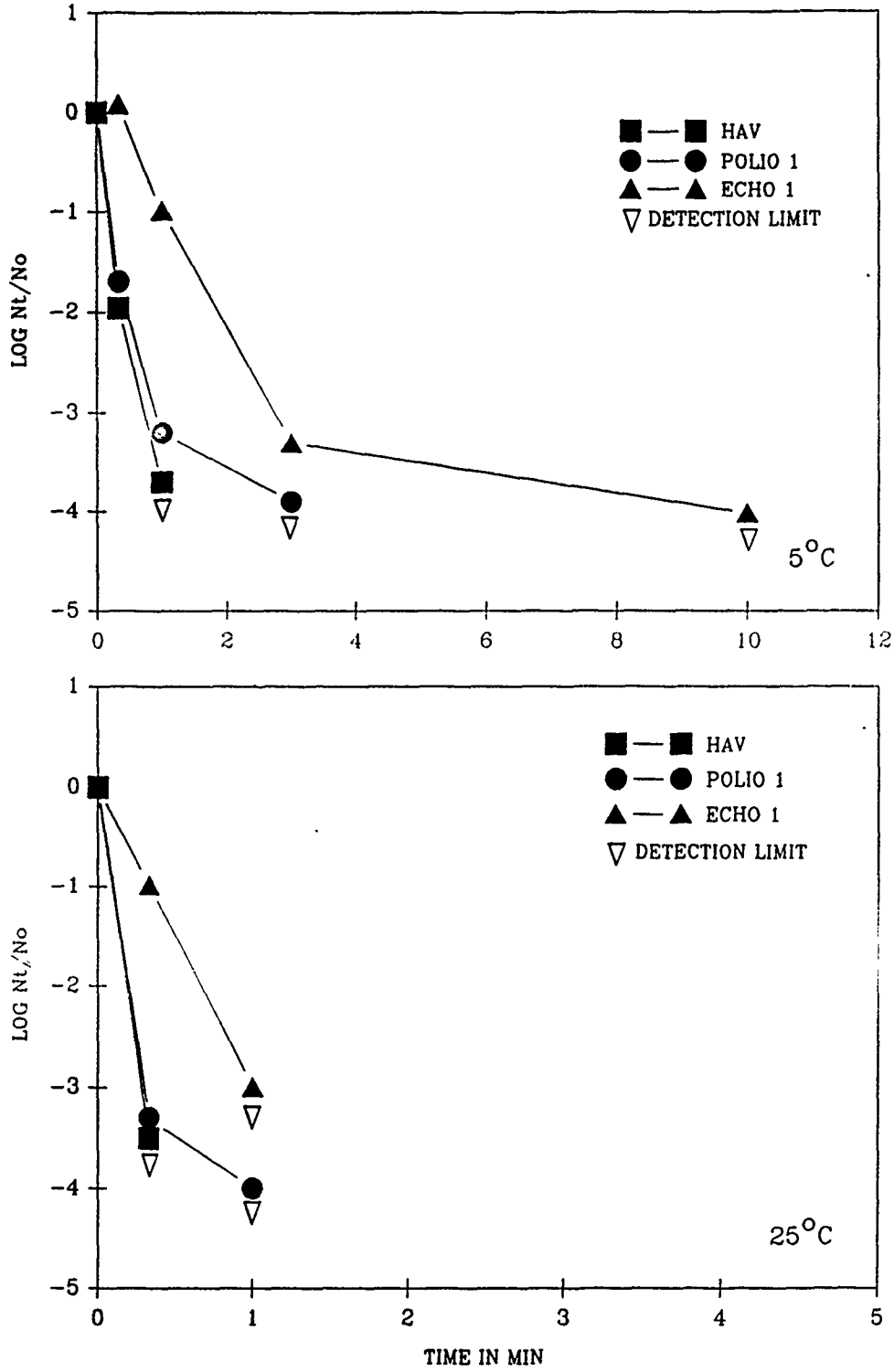


Figure 5. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 1.0 mg/L at pH 7.0, 5°C and 25°C in halogen demand free water

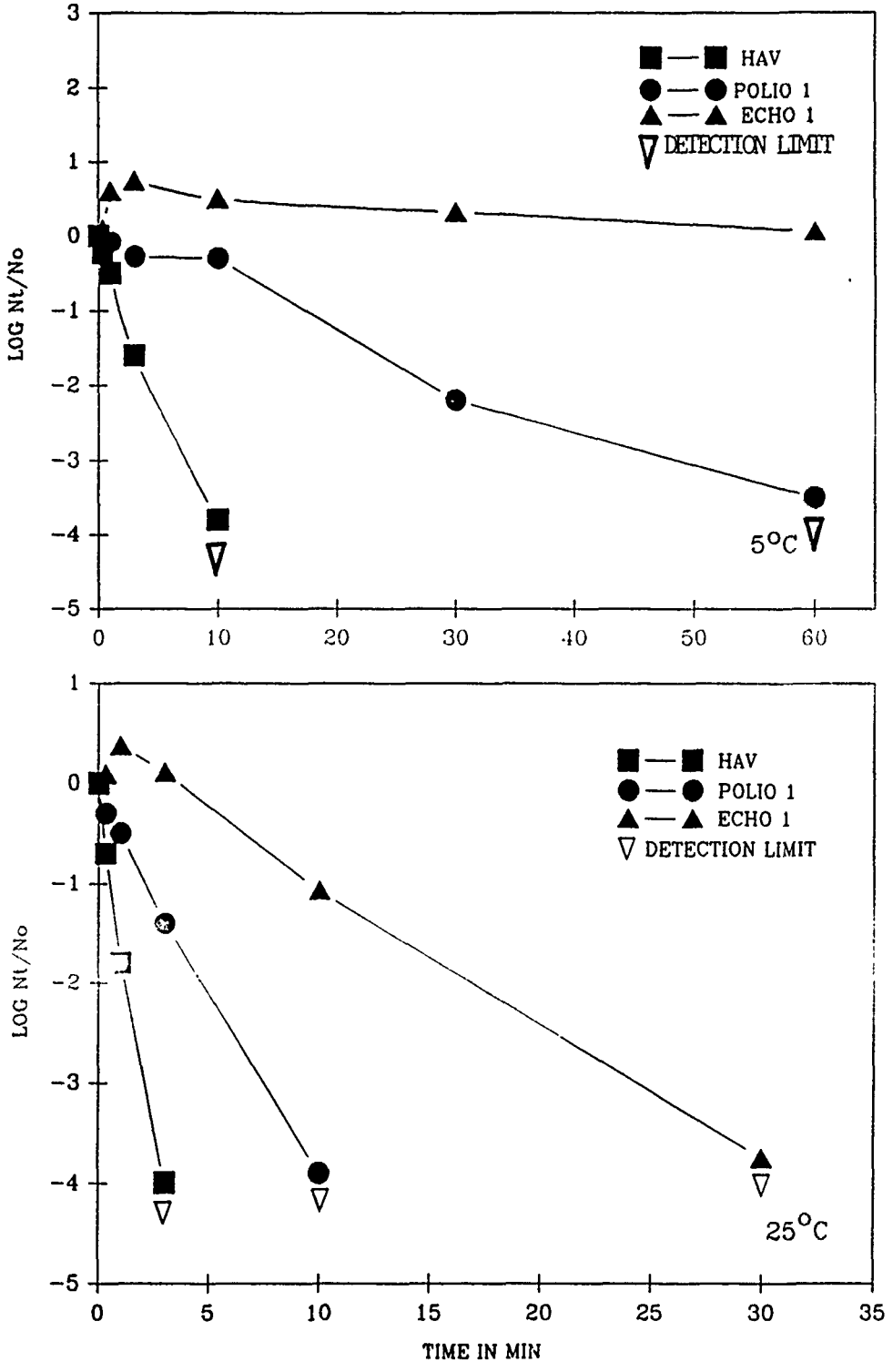


Figure 6. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 1.0 mg/L at pH 9.5, 5°C and 25°C in halogen demand free water.

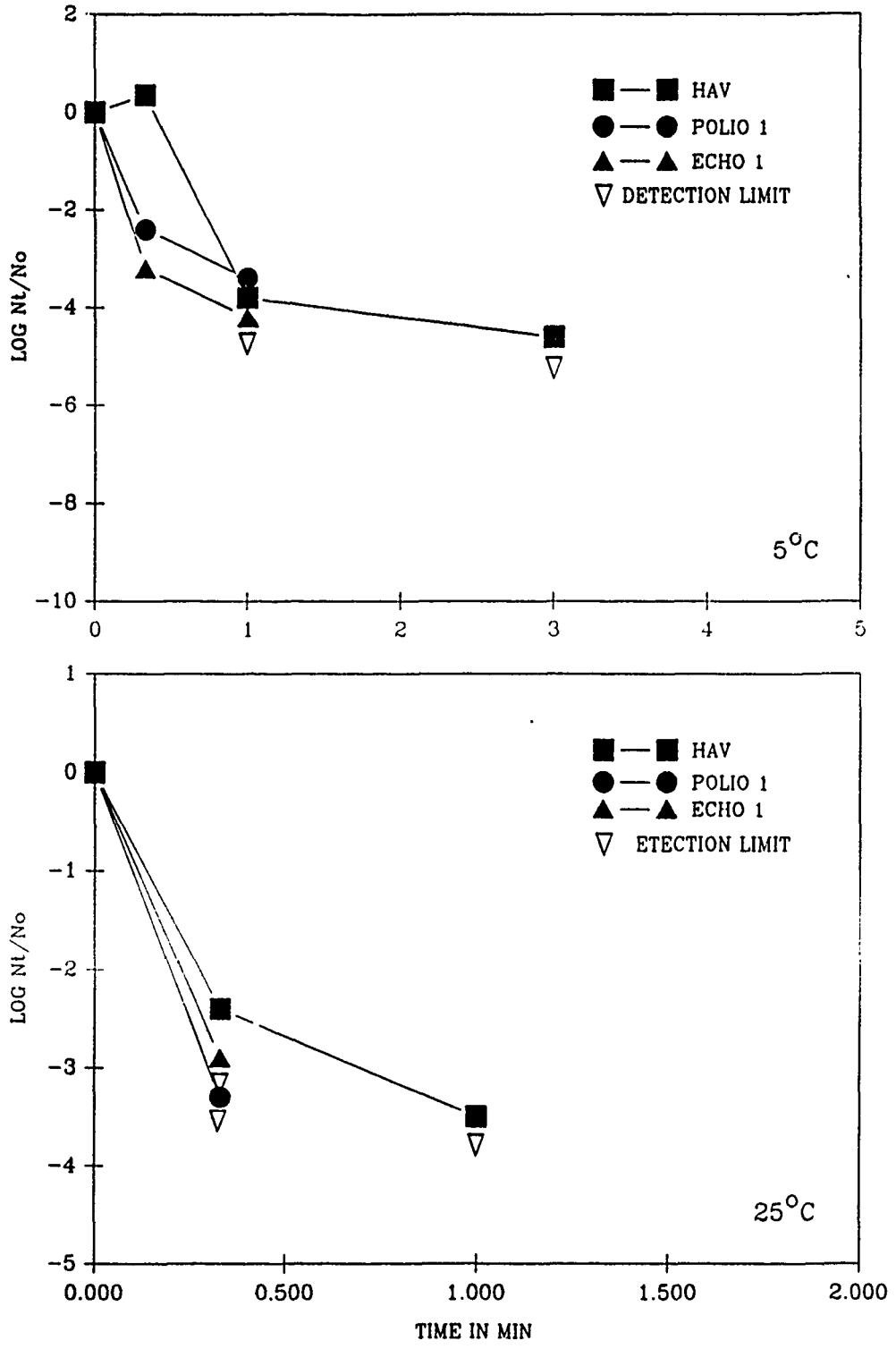


Figure 7. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 5.0 mg/L at pH 4.5, 5°C and 25°C in halogen demand free water.

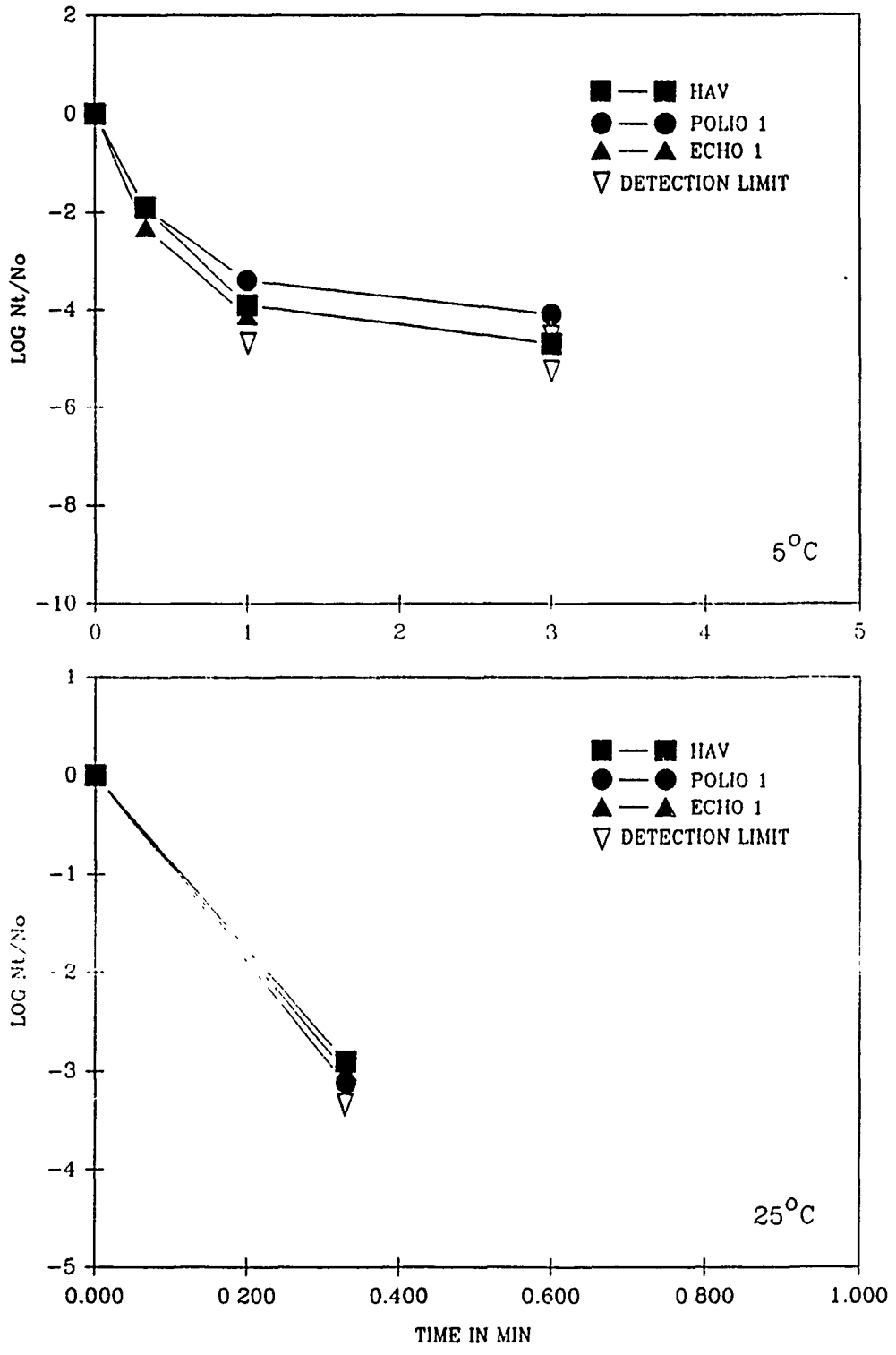


Figure 8. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 5.0 mg/L at pH 7.0, 5°C and 25°C in halogen demand free water.

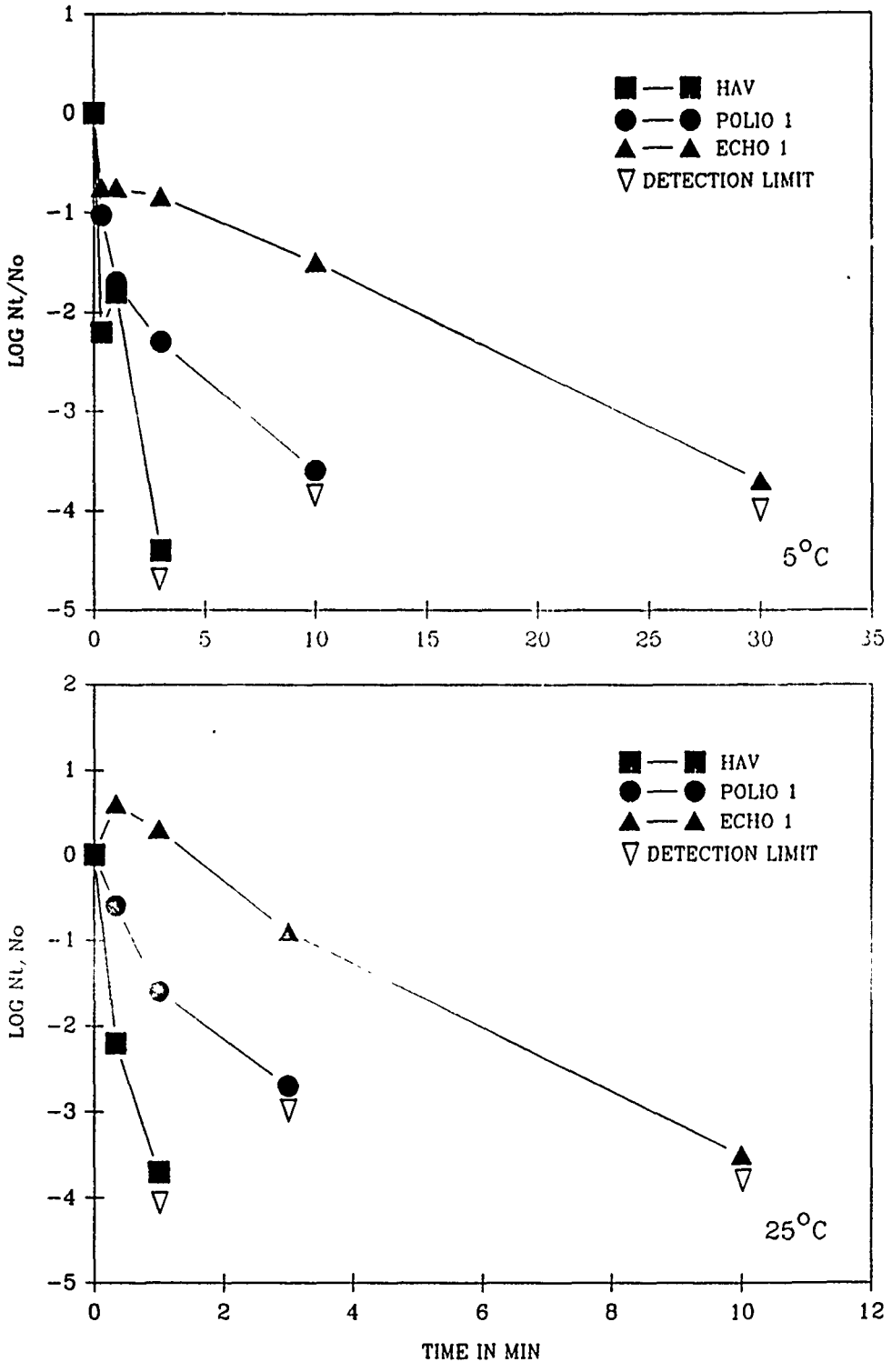


Figure 9. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 5.0 mg/L at pH 9.5, 5°C and 25°C in halogen demand free water.

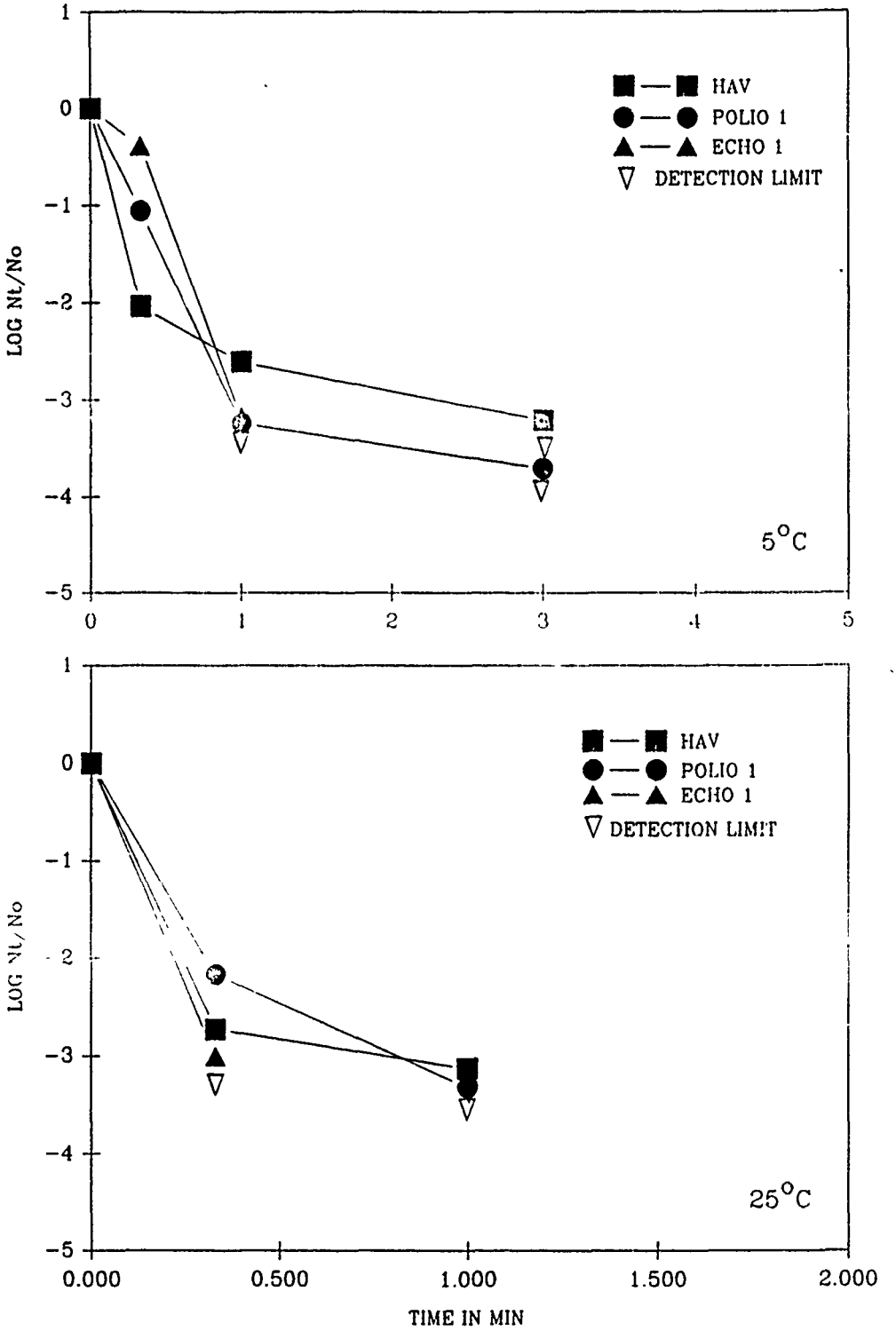


Figure 10. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 3.0 mg/L at pH 4.5, 5°C and 25°C in worst case water.

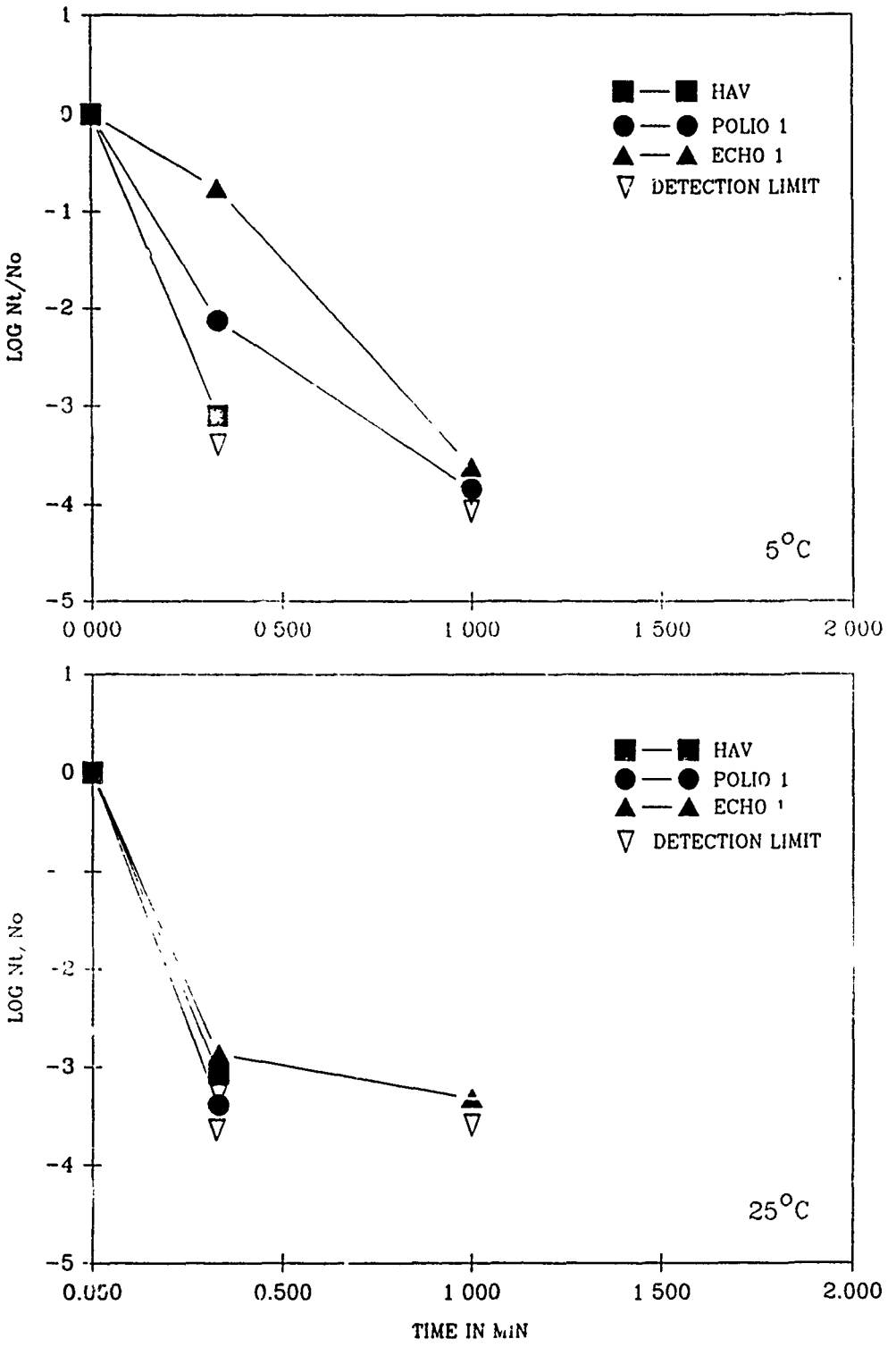


Figure 11. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 3.0 mg/L at pH 7.0, 5°C and 25°C in worst case water.

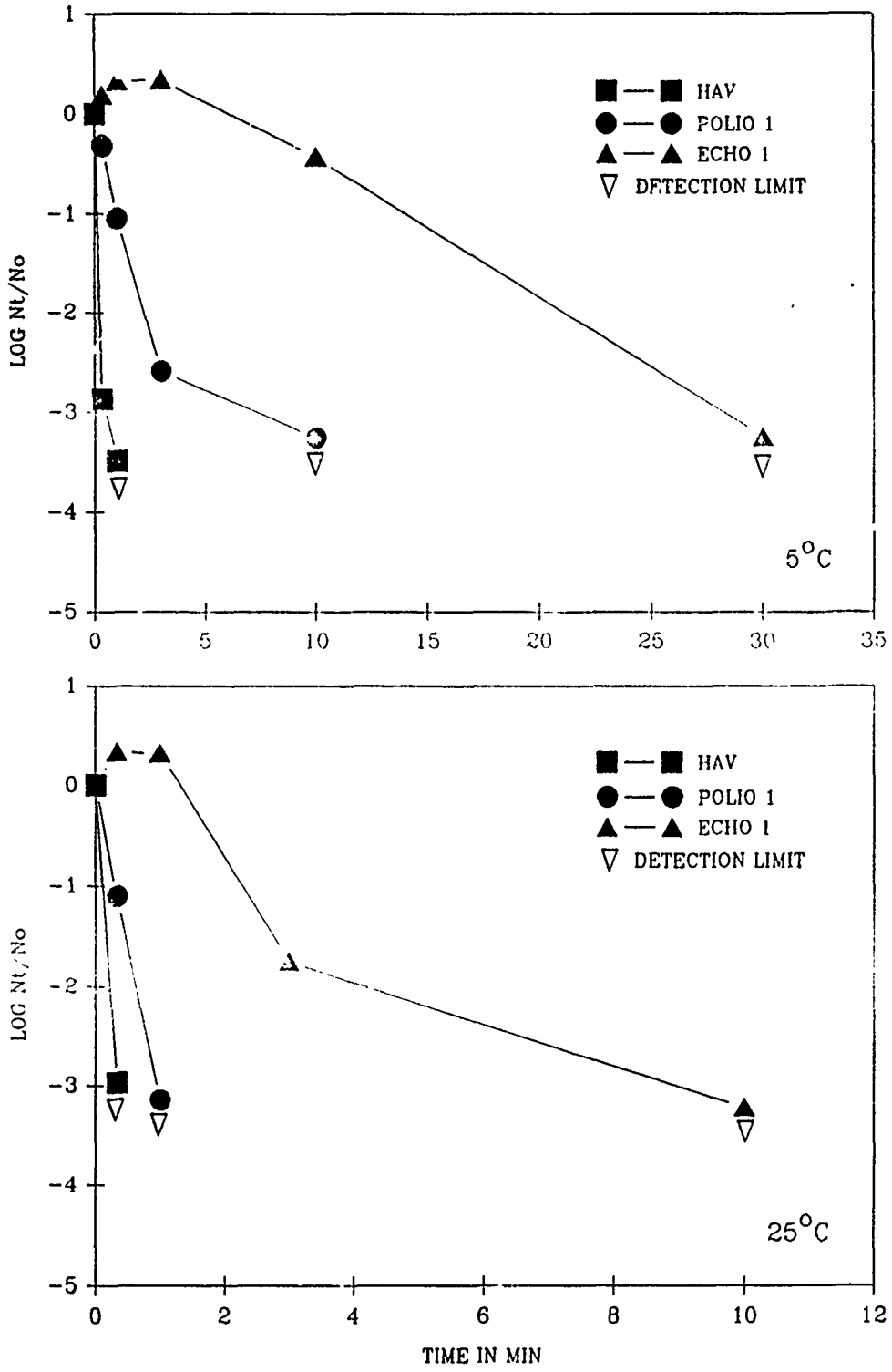


Figure 12. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 3.0 mg/L at pH 9.5, 5°C and 25°C in worst case water.

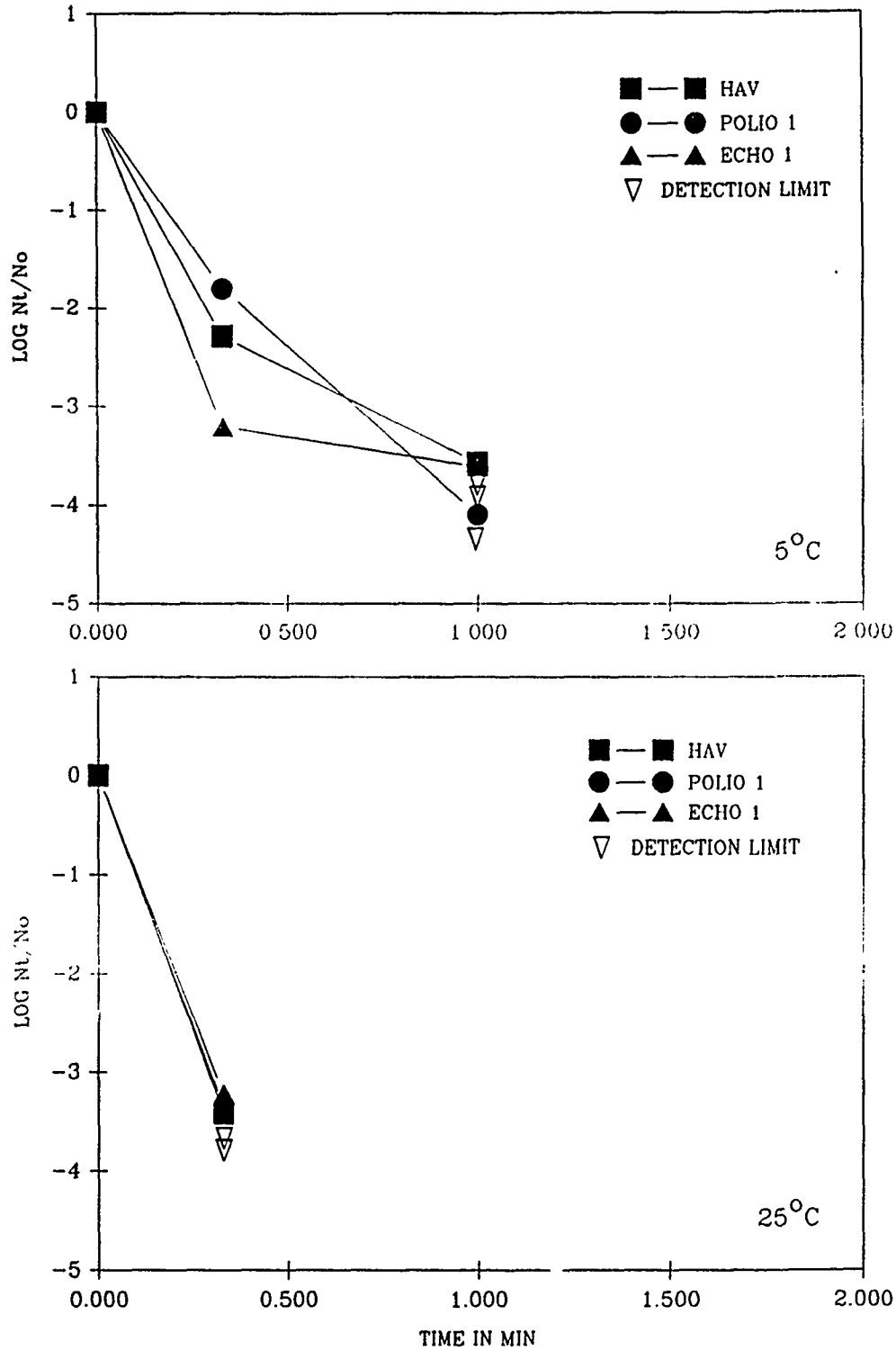


Figure 13. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 7.0 mg/L at pH 4.5, 5°C and 25°C in worst case water.

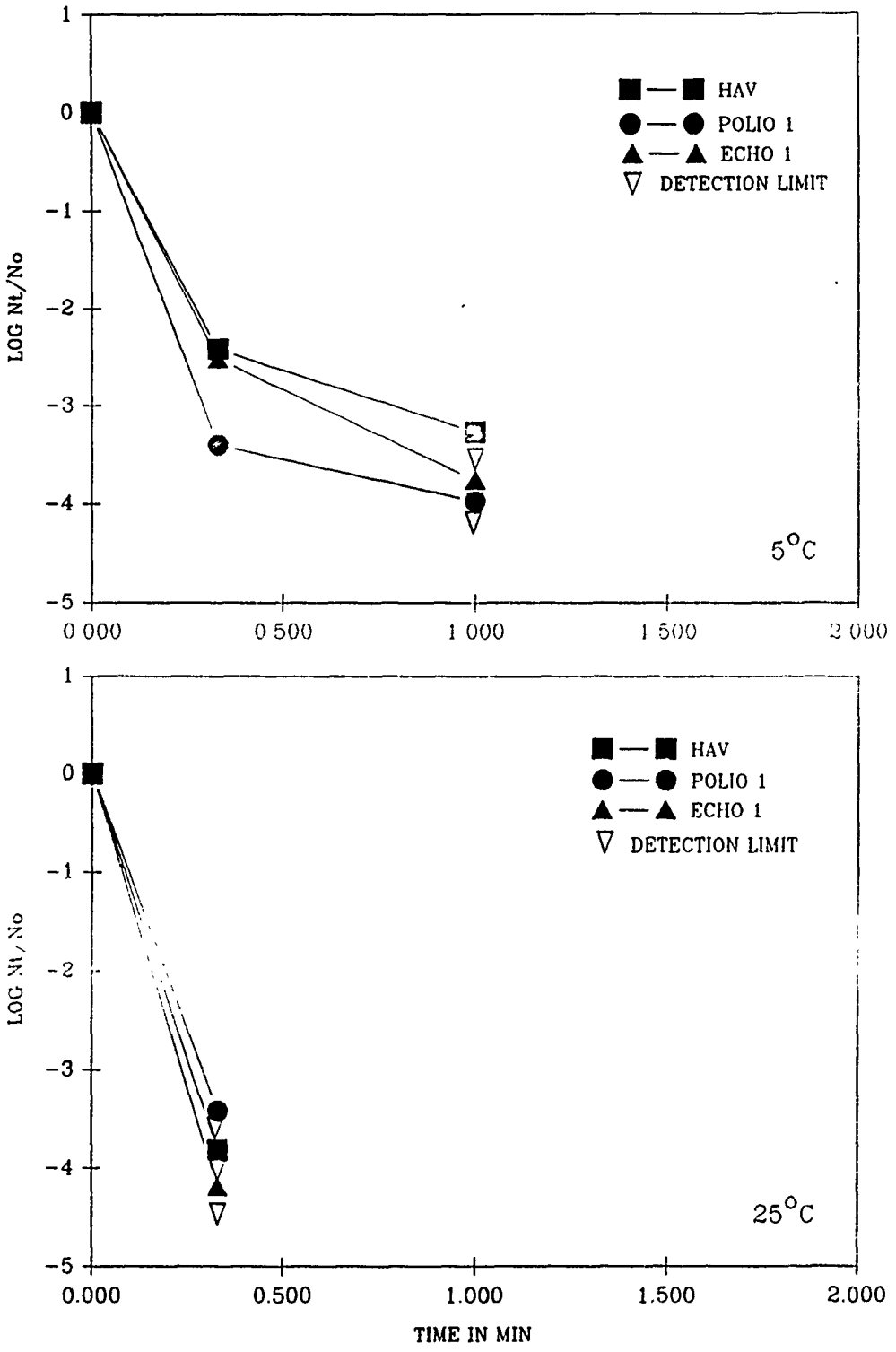


Figure 14. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 7.0 mg/L at pH 7.0, 5°C and 25°C in worst case water.

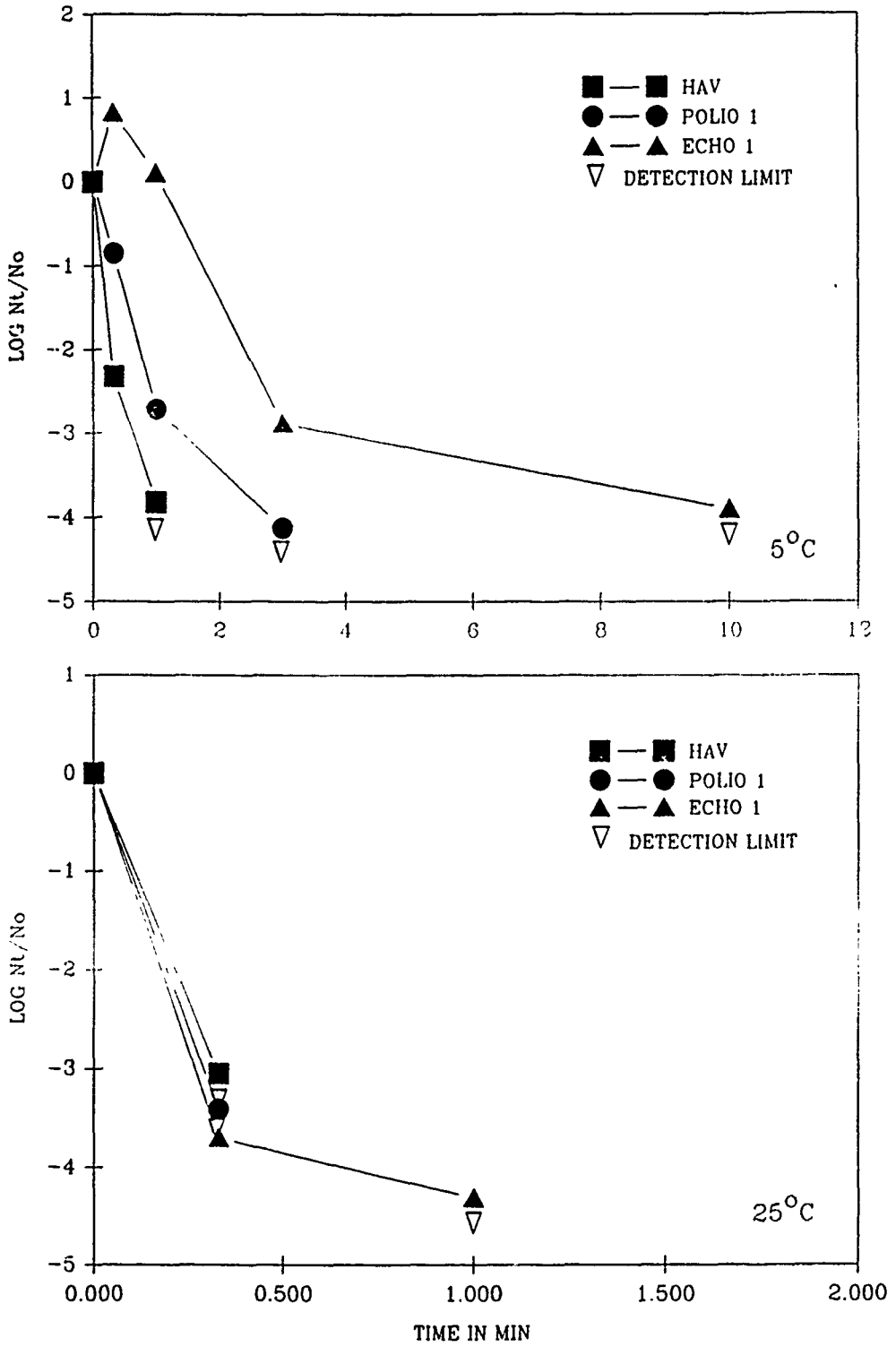


Figure 15. Inactivation of HAV, poliovirus 1 and echovirus 1 by an initial free chlorine dose of approximately 7.0 mg/L at pH 9.5, 5°C and 25°C in worst case water.

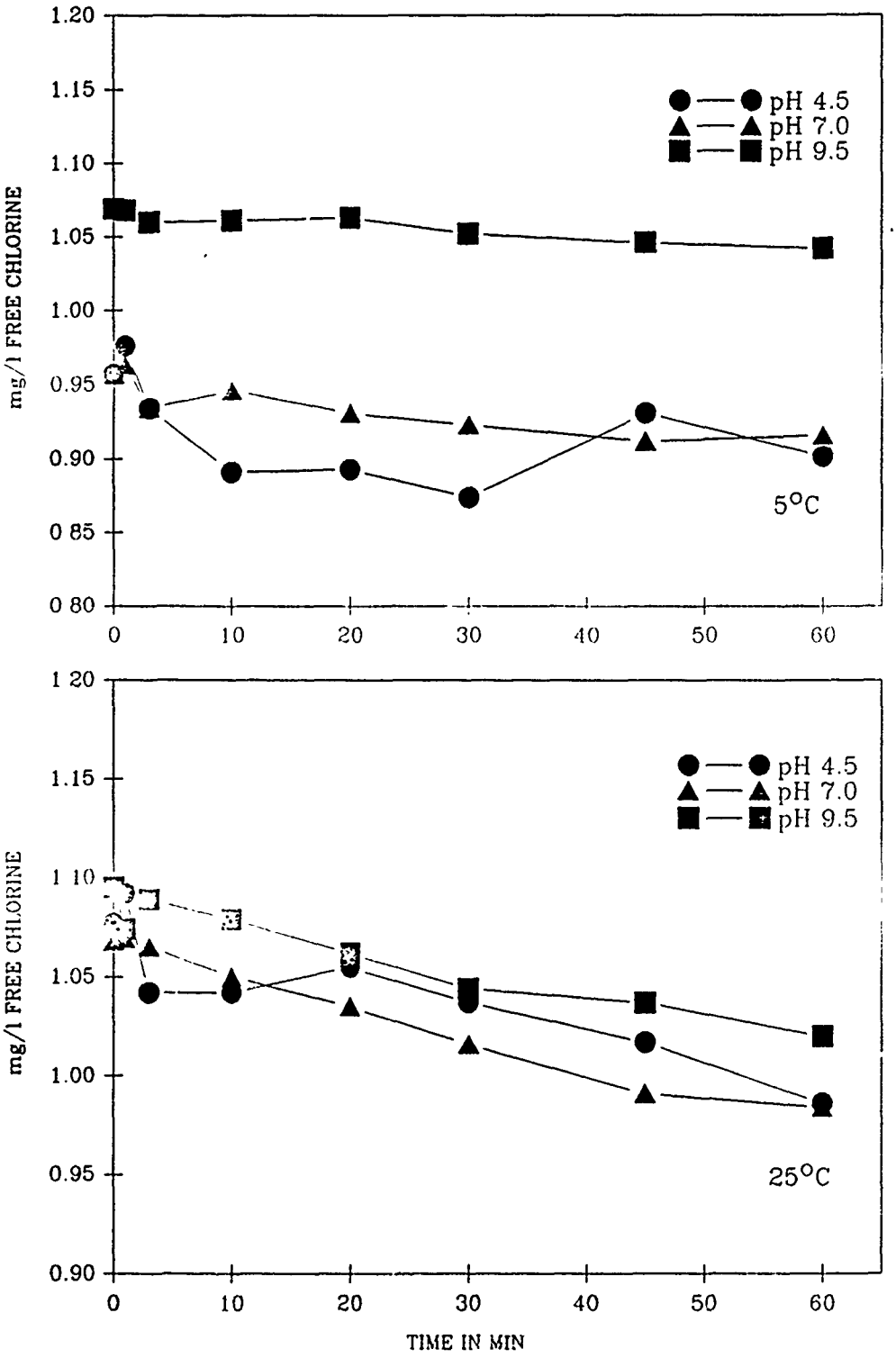


Figure 16. Free chlorine decay curves at an initial free chlorine concentration of approximately 1.0 mg/l at pH 4.5, 7.0 and 9.5 in halogen demand free water at 5°C and 25°C.

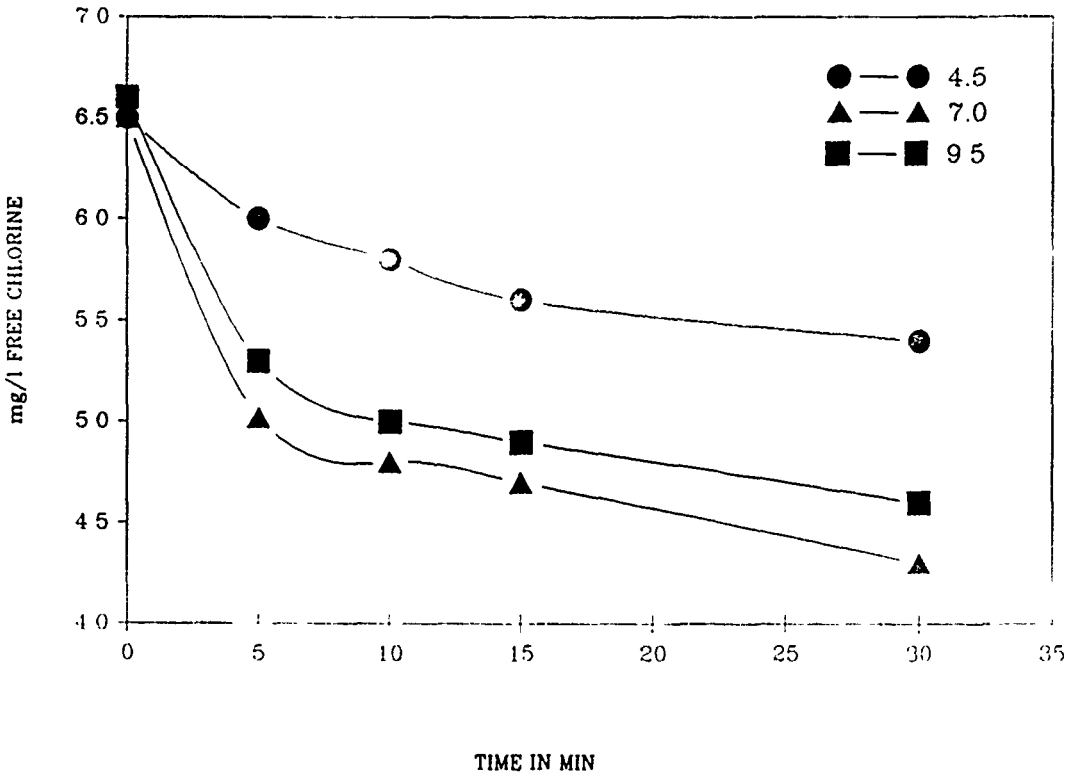


Figure 17. Free chlorine decay curves at an initial free chlorine concentration of approximately 6.5 mg/l at pH 4.5, 7.0 and 9.5 and 5°C in worst case water.

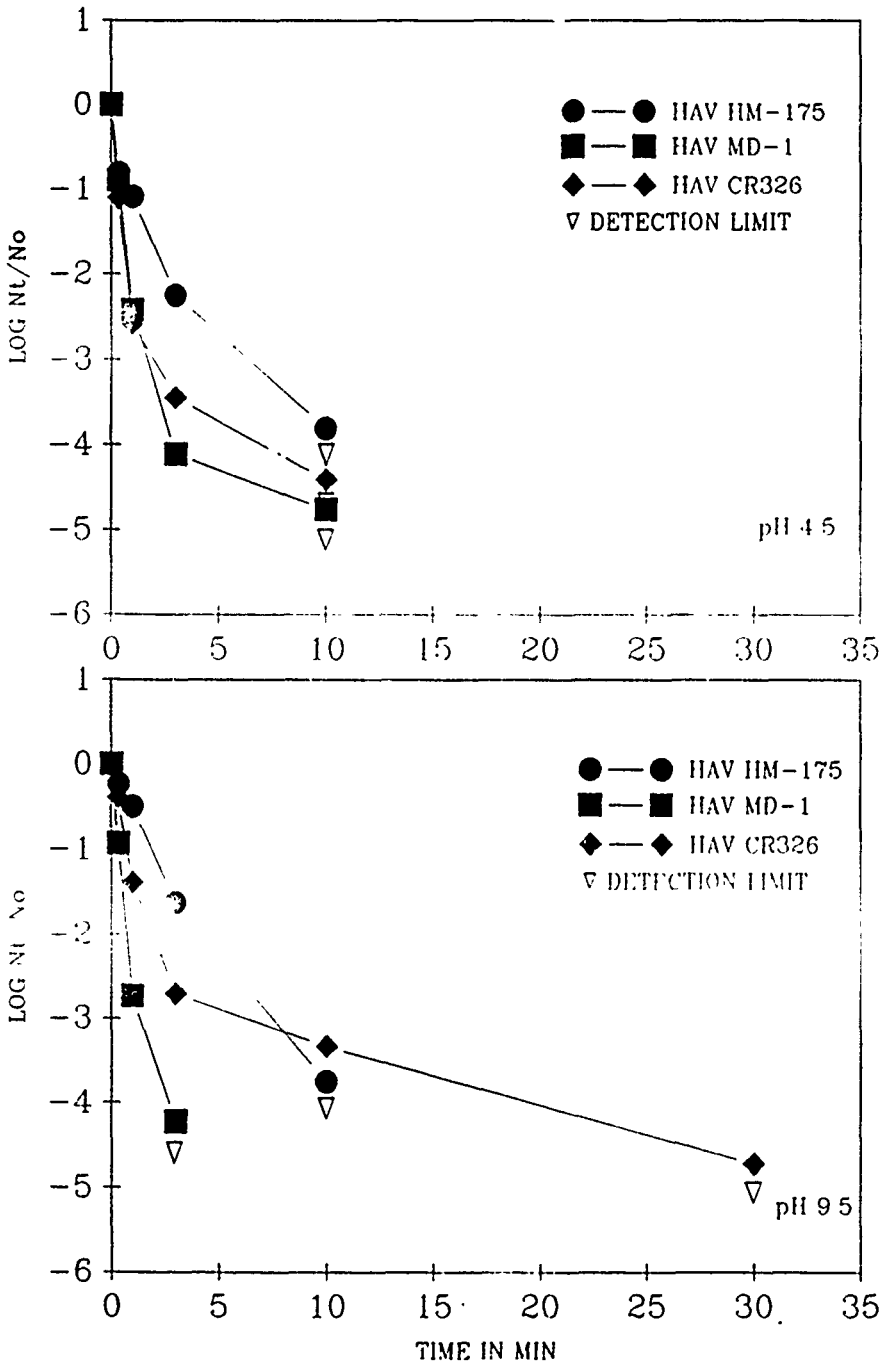


FIGURE 18. INACTIVATION OF HAV STRAINS HM175, MD-1 AND CR326 BY A 1 MG/L DOSE OF FREE CHLORINE IN BUFFERED DEMAND FREE WATER AT pH 4.5 AND 9.5 AND 5°C

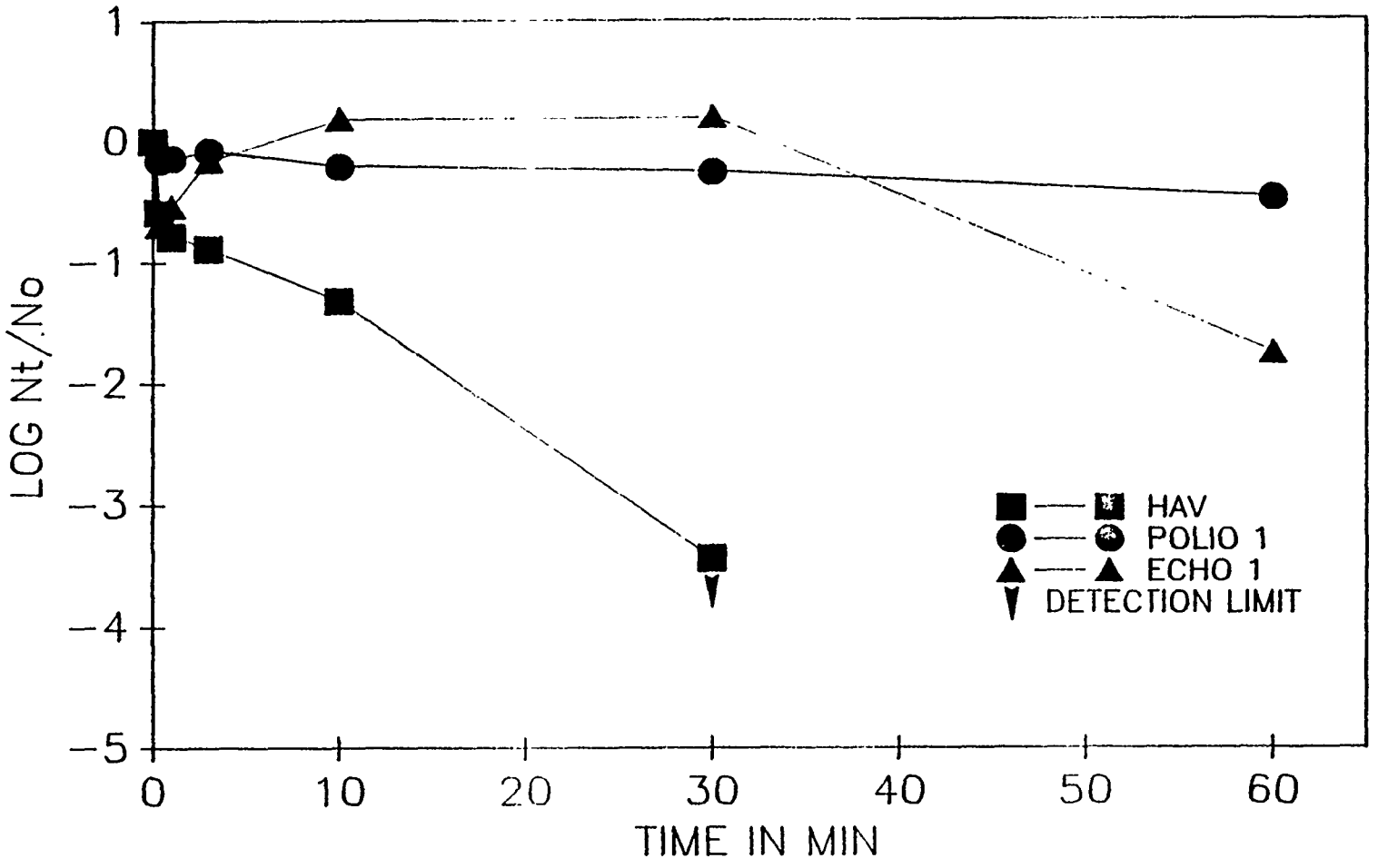


FIGURE 19. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 4.5, 5°C

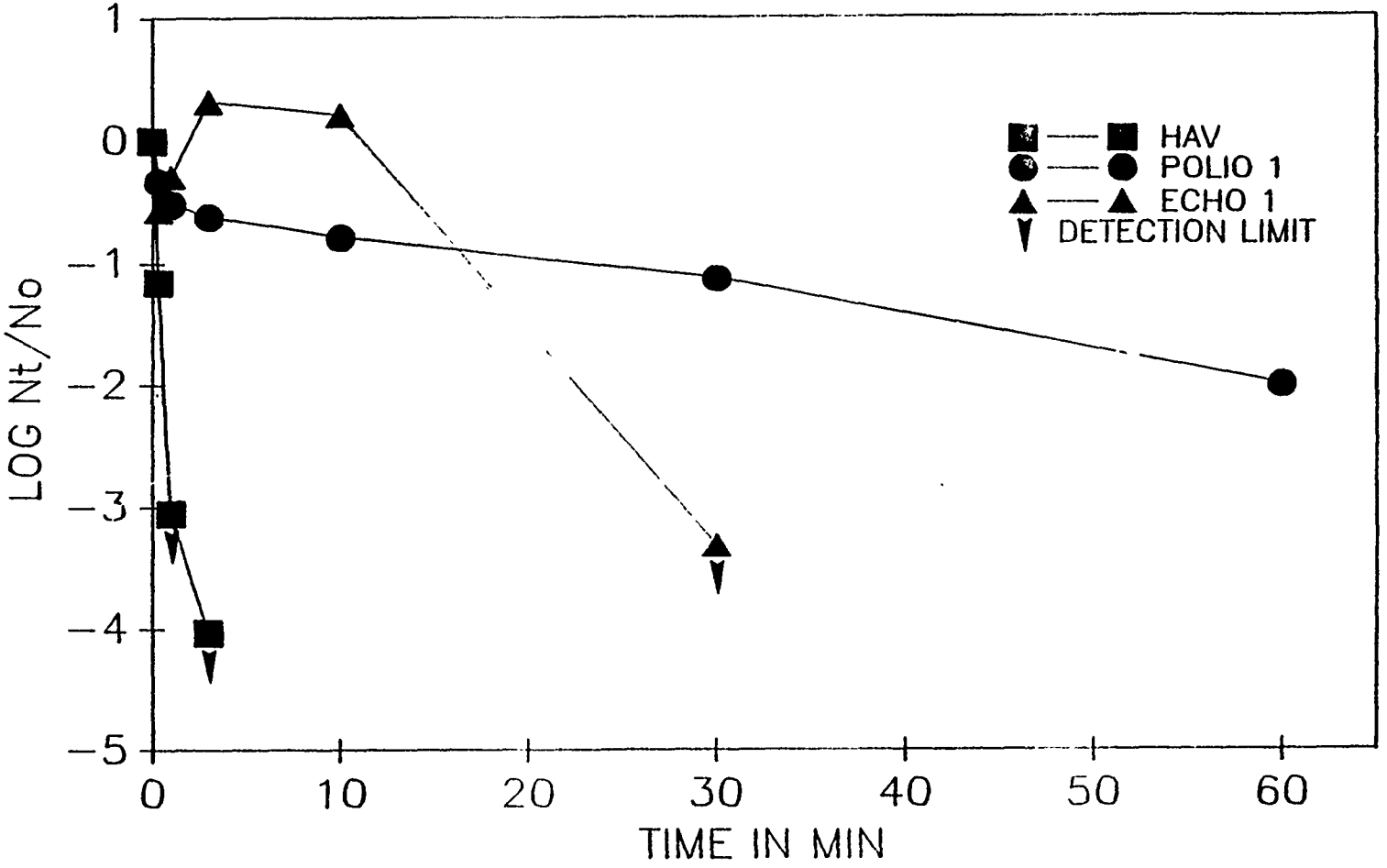


FIGURE 20. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 7.0, 5°C

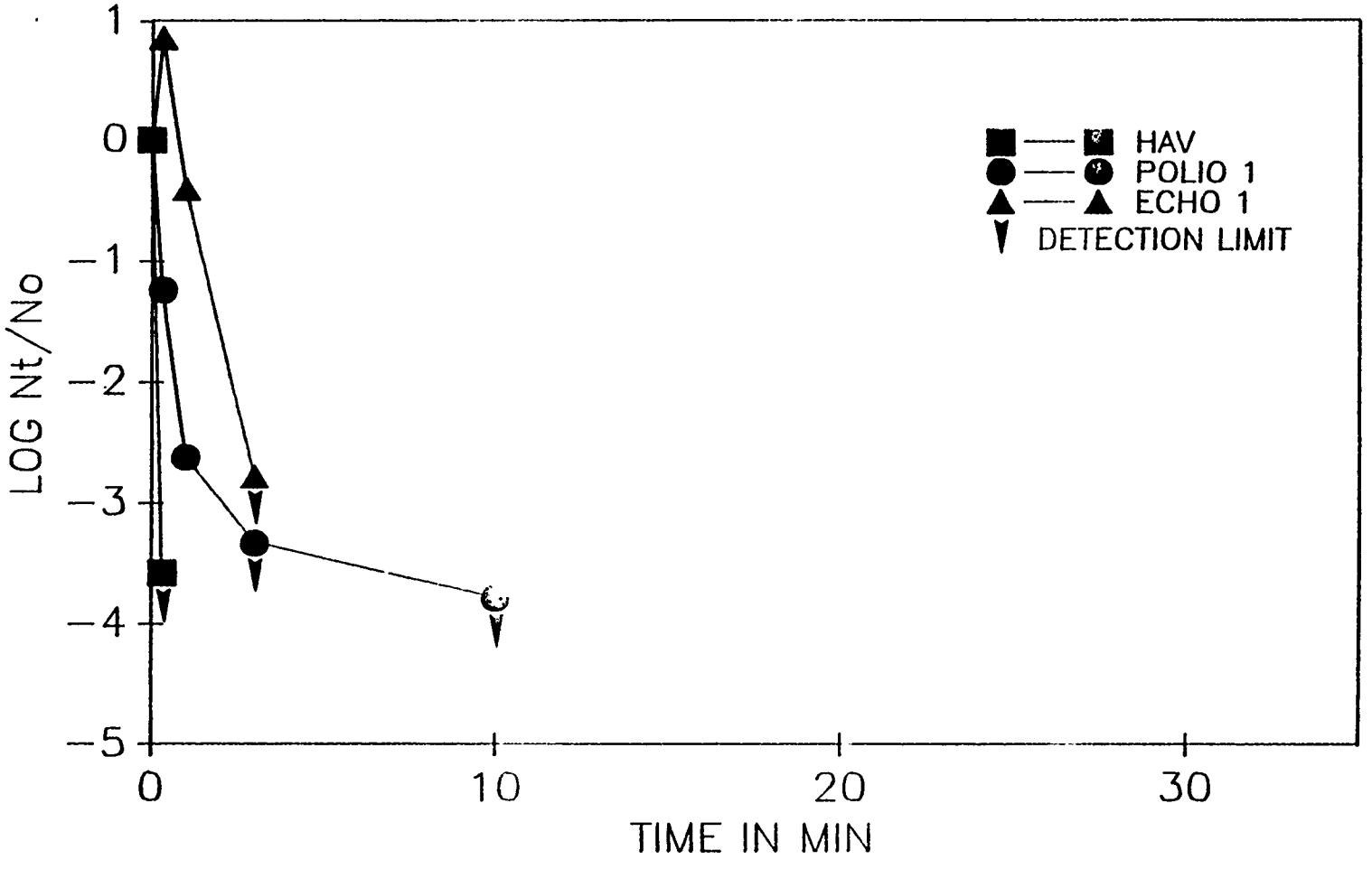


FIGURE 21. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 9.5, 5°C

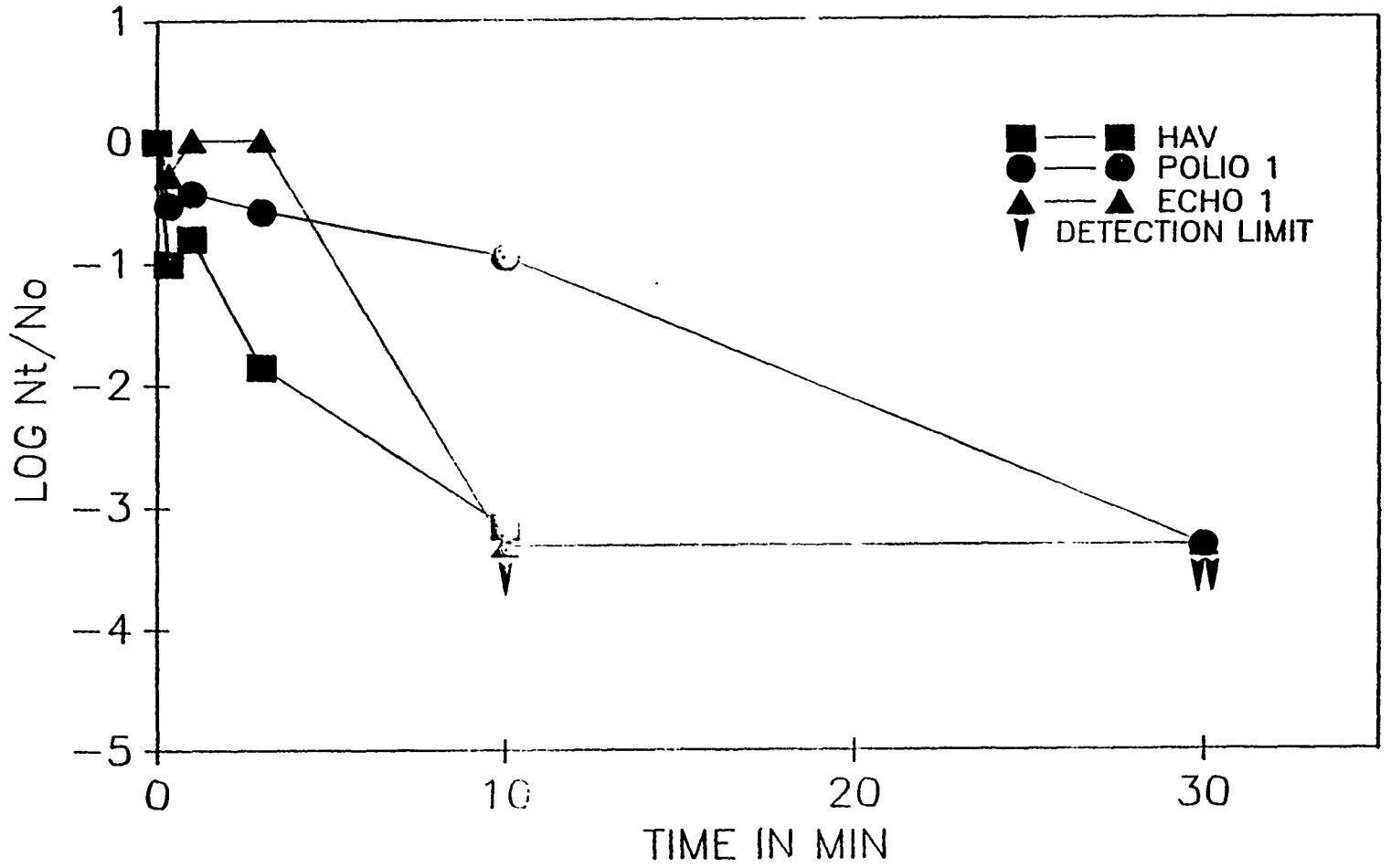


FIGURE 22. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 4.5, 25°C

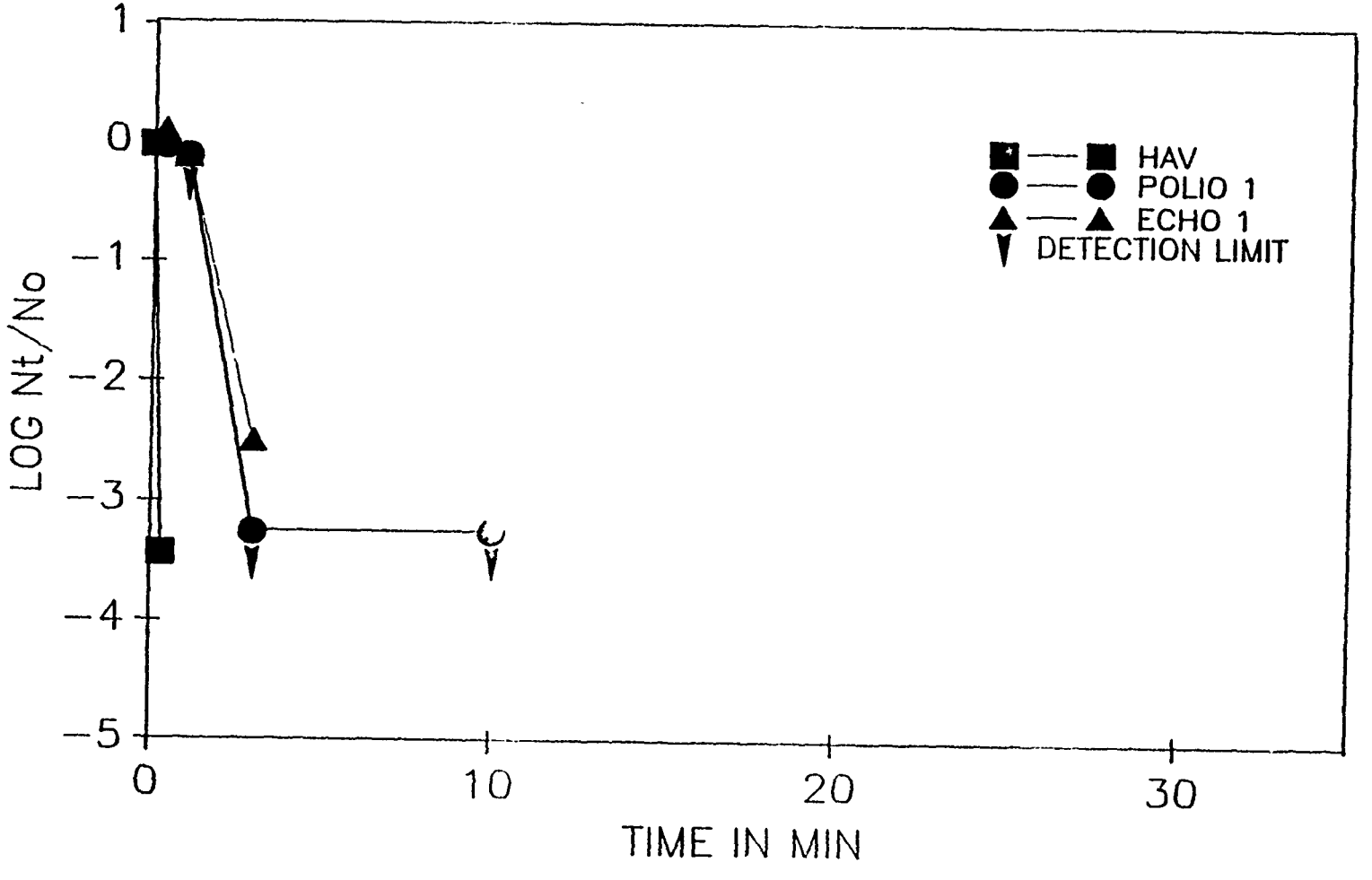


FIGURE 23. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 7.0, 25°C

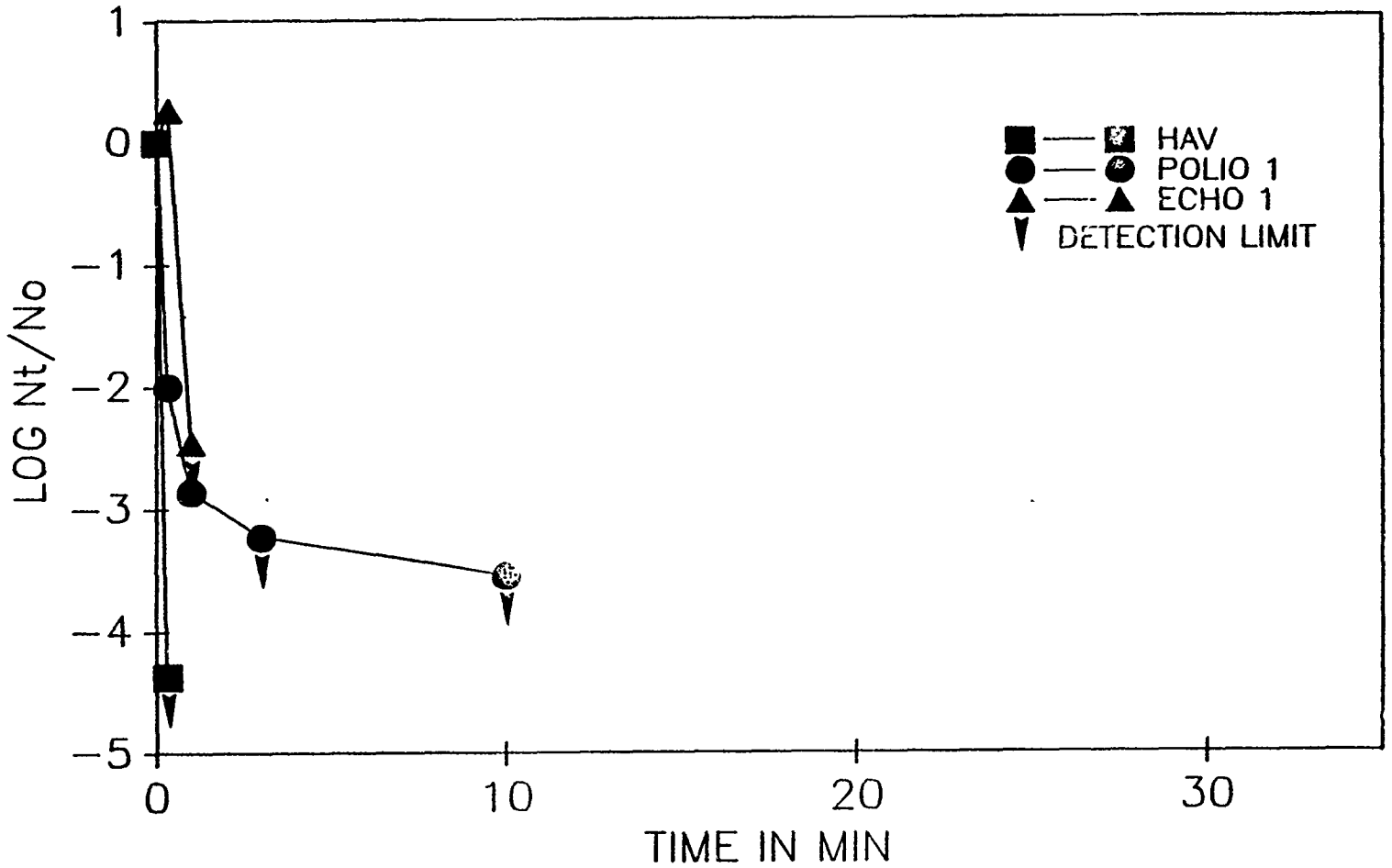


FIGURE 24. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 9.5, 25°C

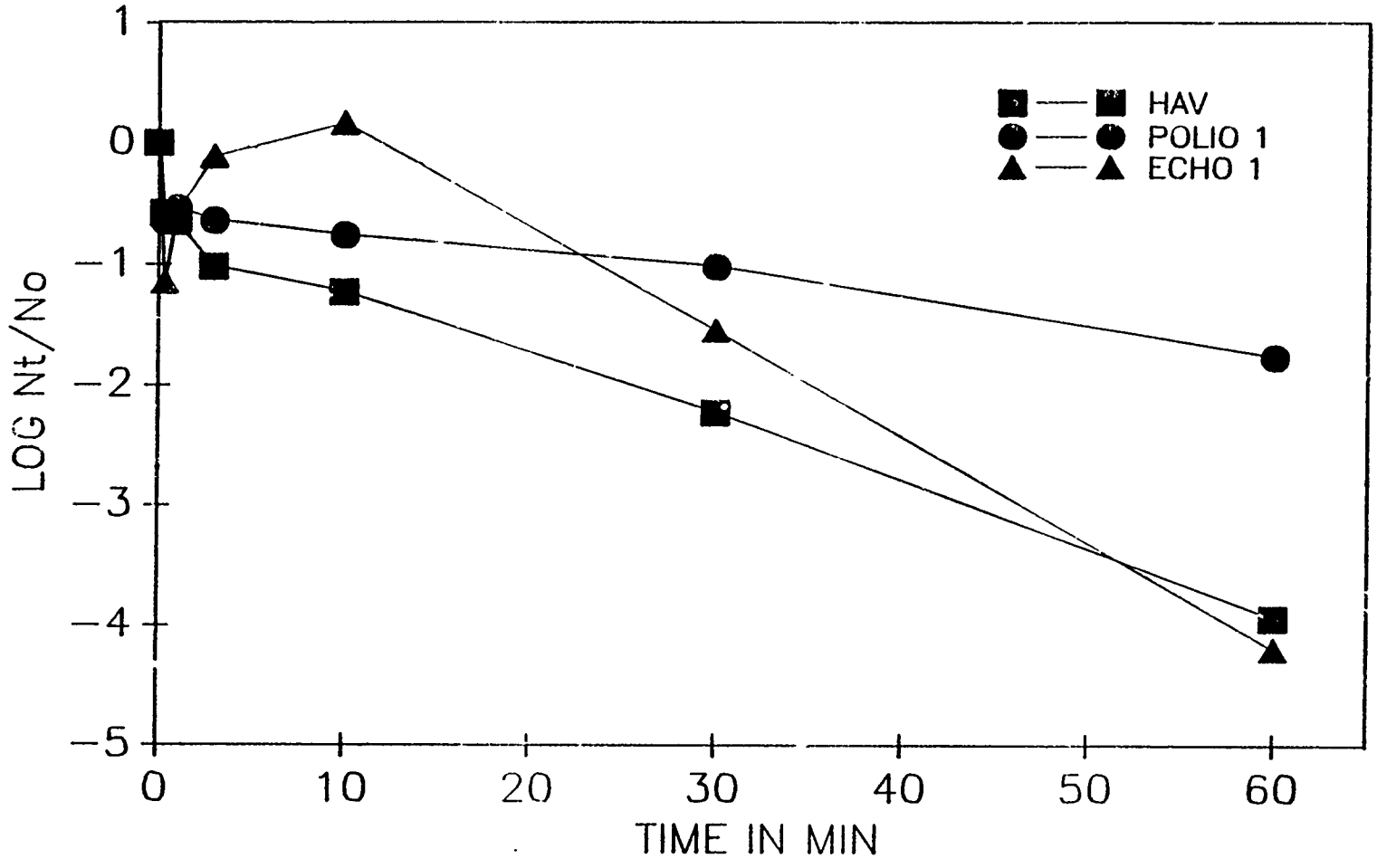


FIGURE 25. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 4.5, 5°C

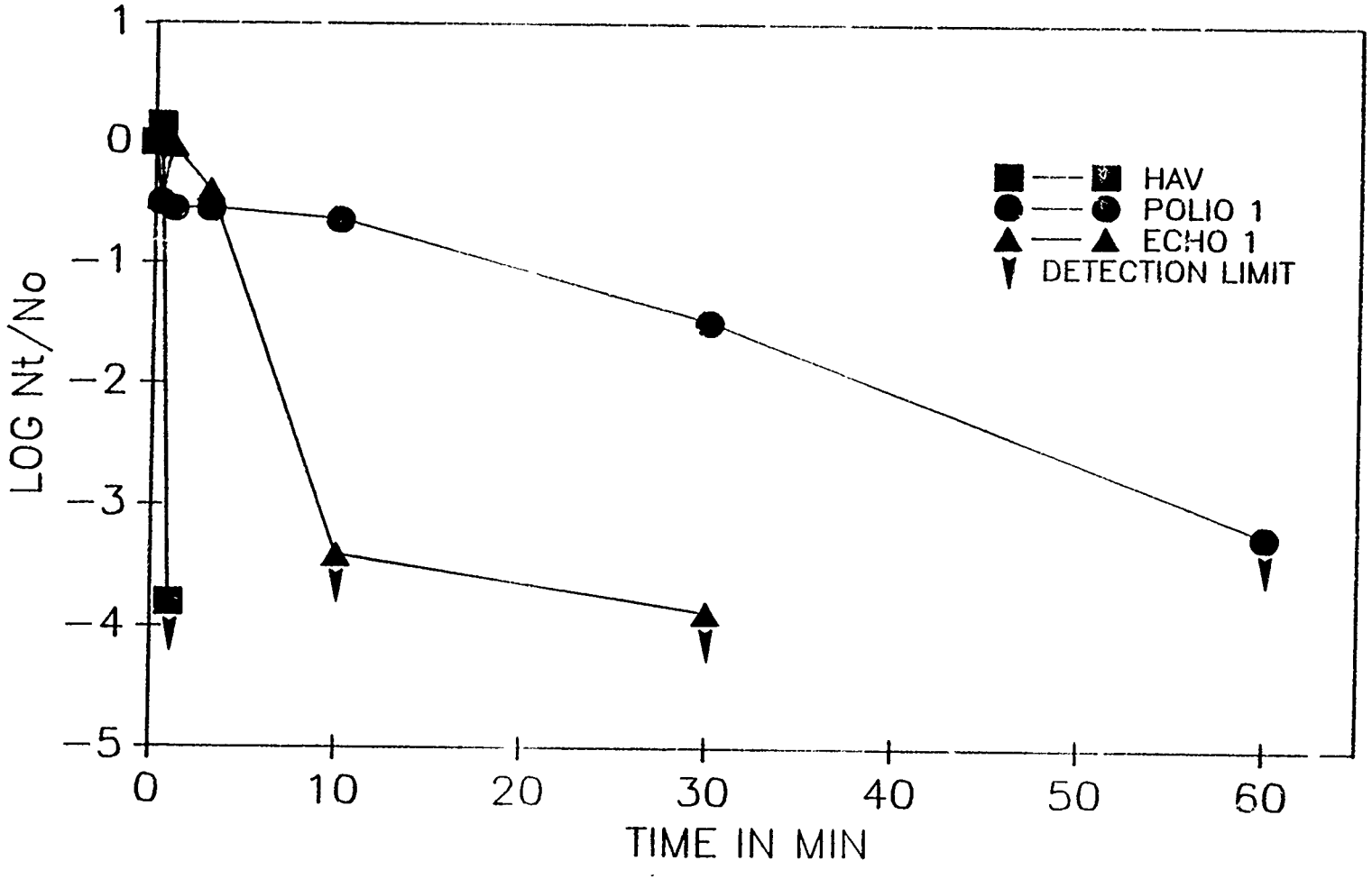


FIGURE 26. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 7.0, 5°C

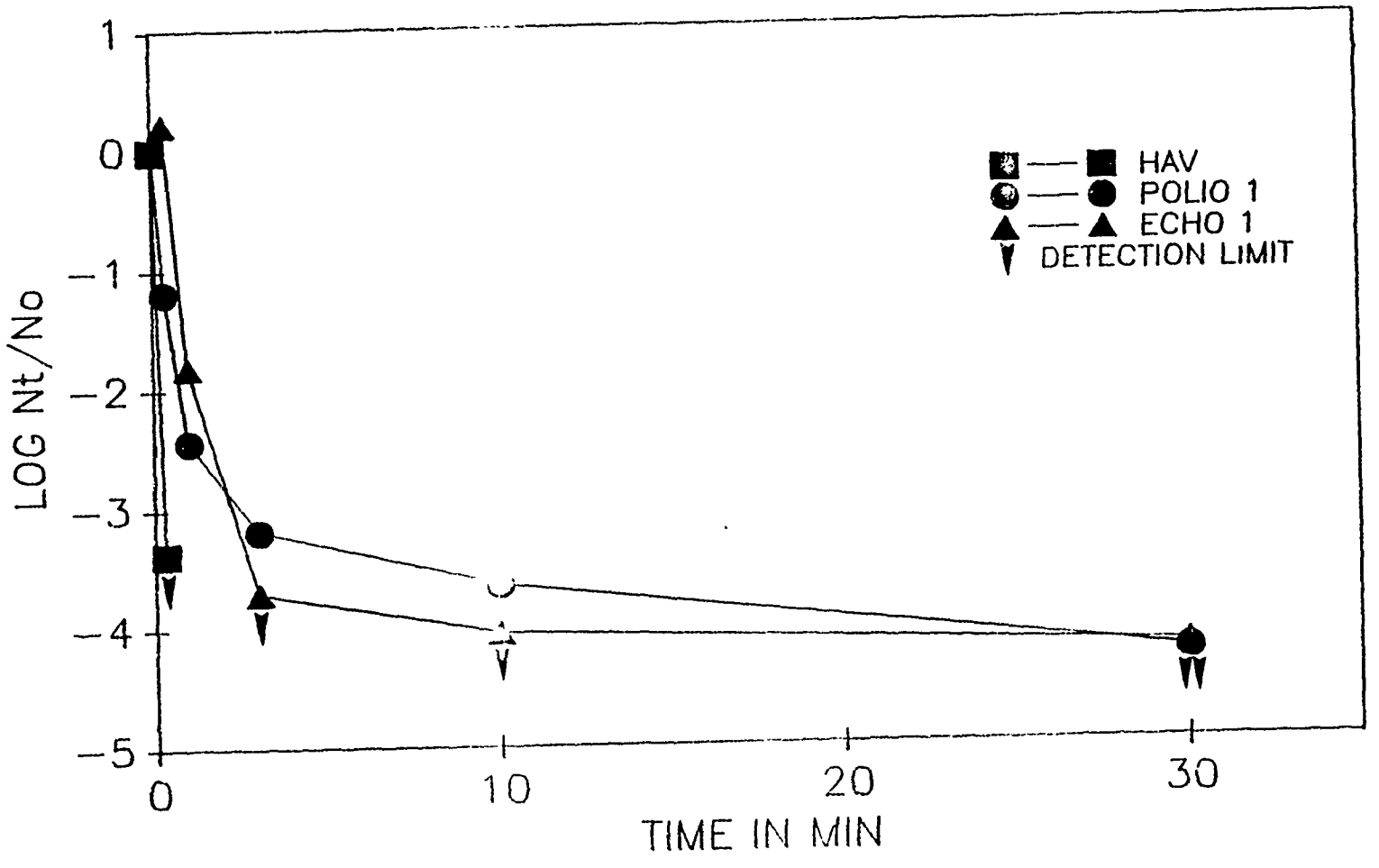


FIGURE 27. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 9.5, 5°C

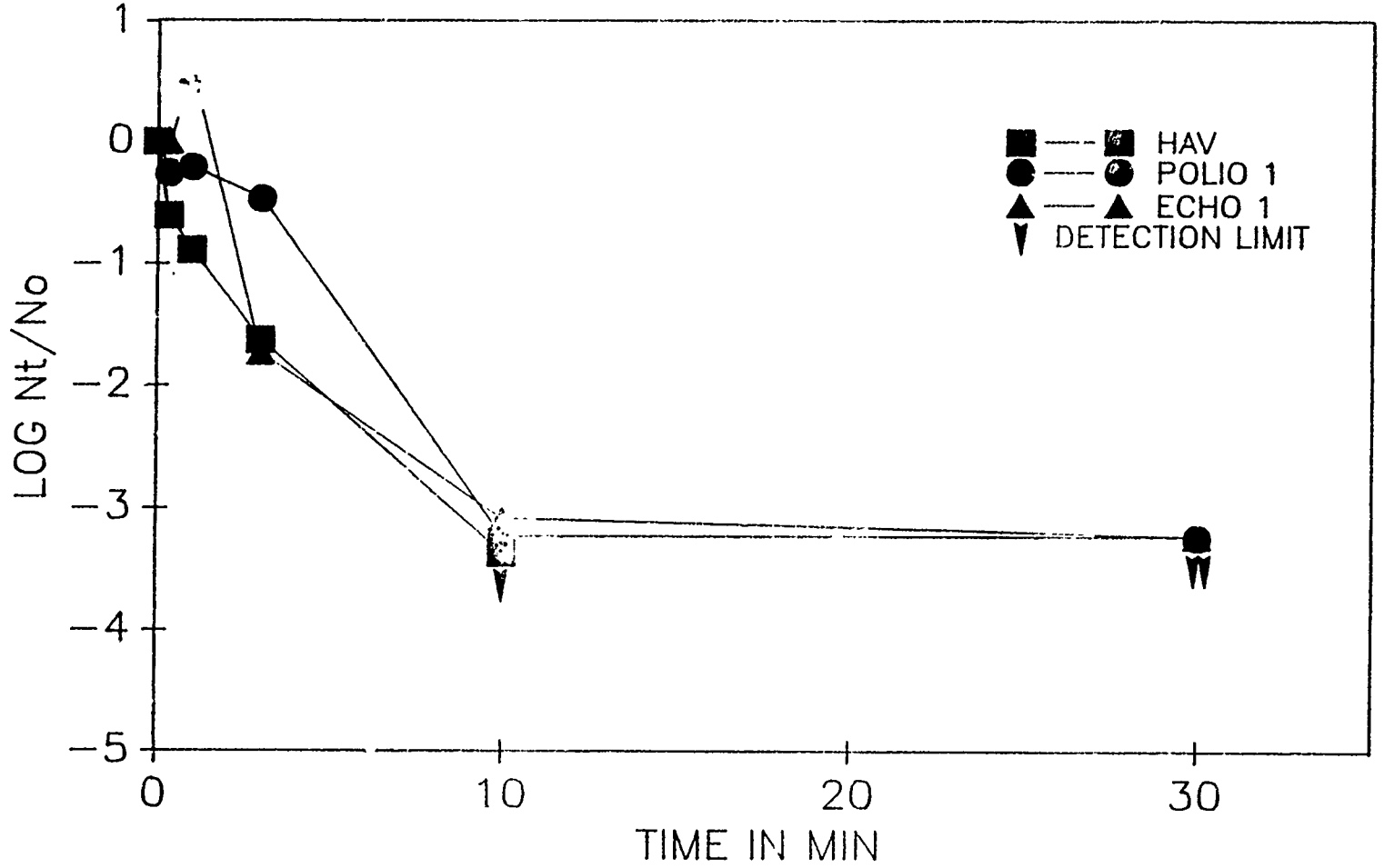


FIGURE 28. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 4.5, 25°C

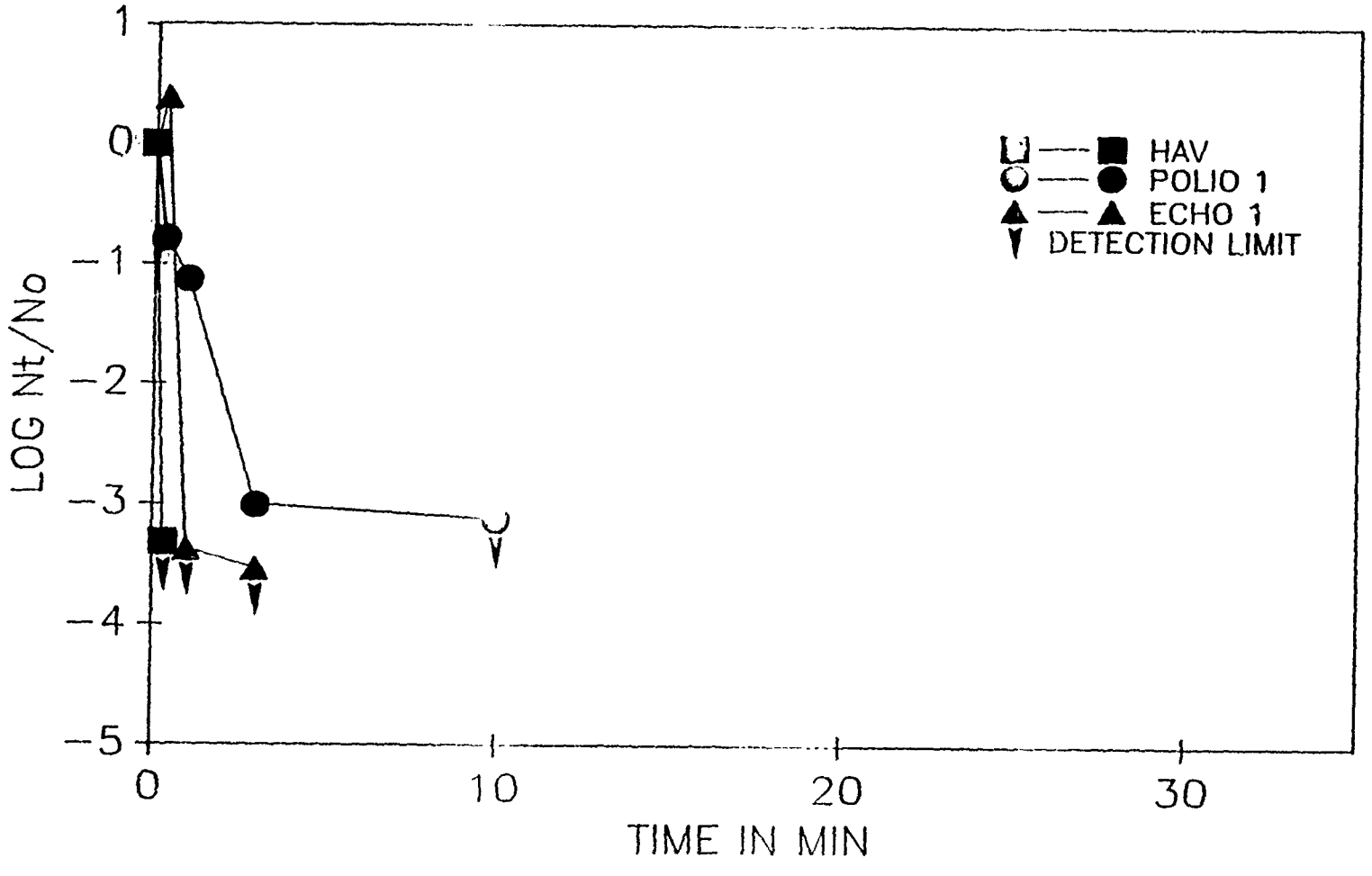


FIGURE 29.. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 7.0, 25°C

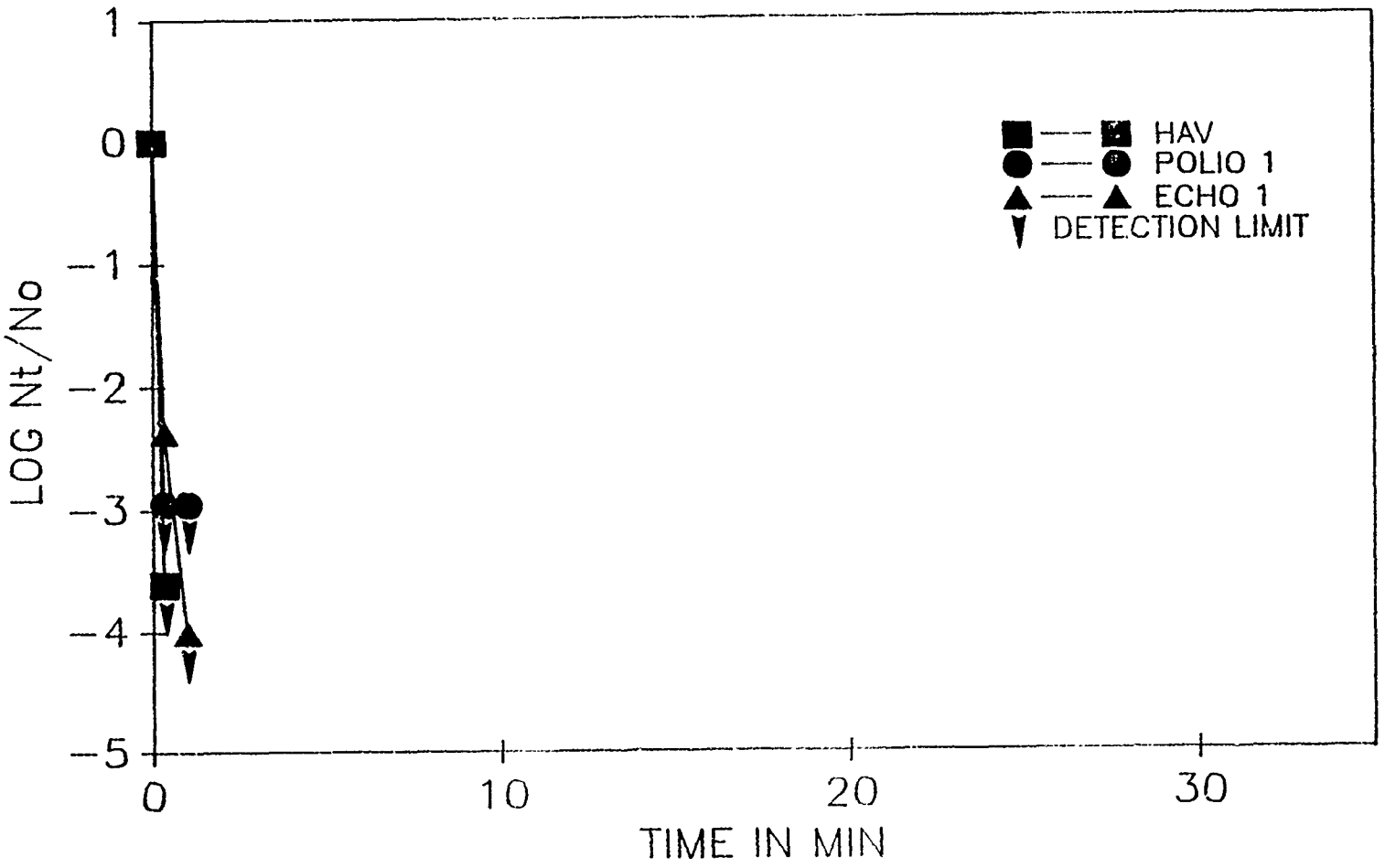


FIGURE 30. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 2 TABLETS/QUART, pH 9.5, 25°C

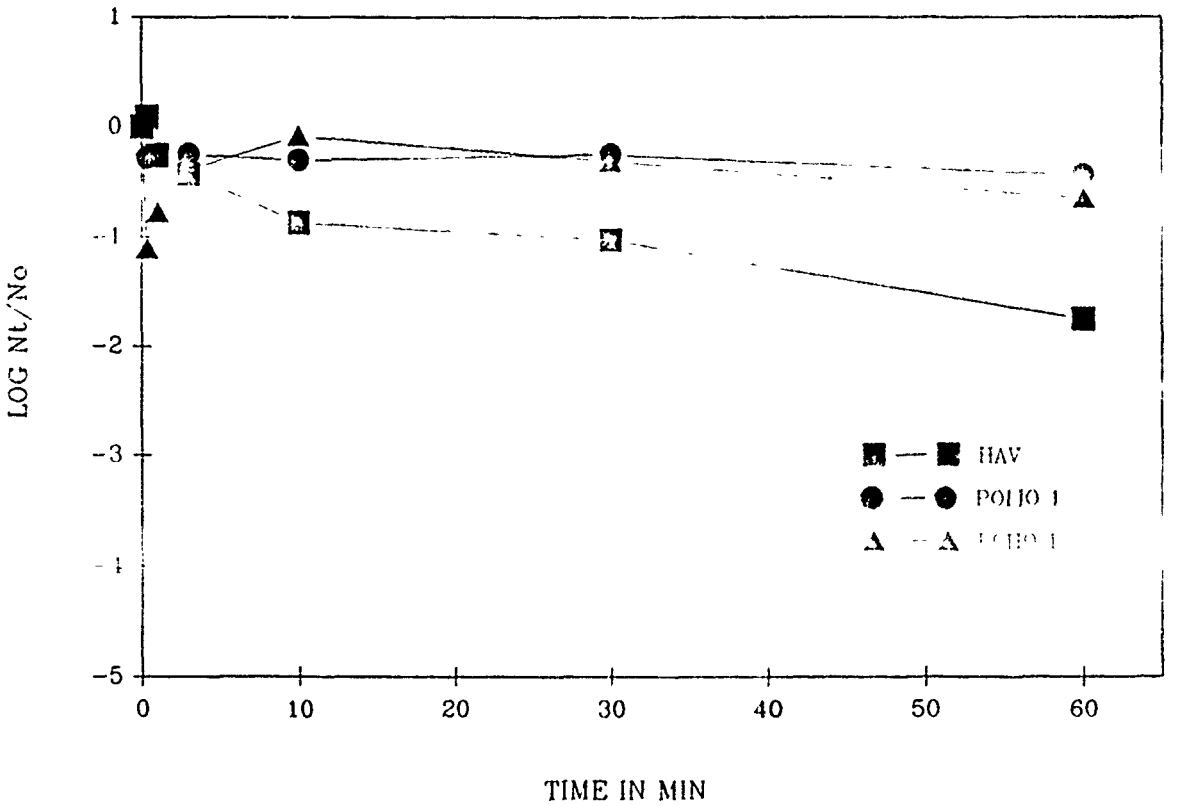


FIGURE 31. INACTIVATION OF HAV, POLIOVIRUS 1 AND ECHOVIRUS 1 BY 1 TABLET PER QUART IN WORST CASE WATER AT pH 4.5 AND 5°C.

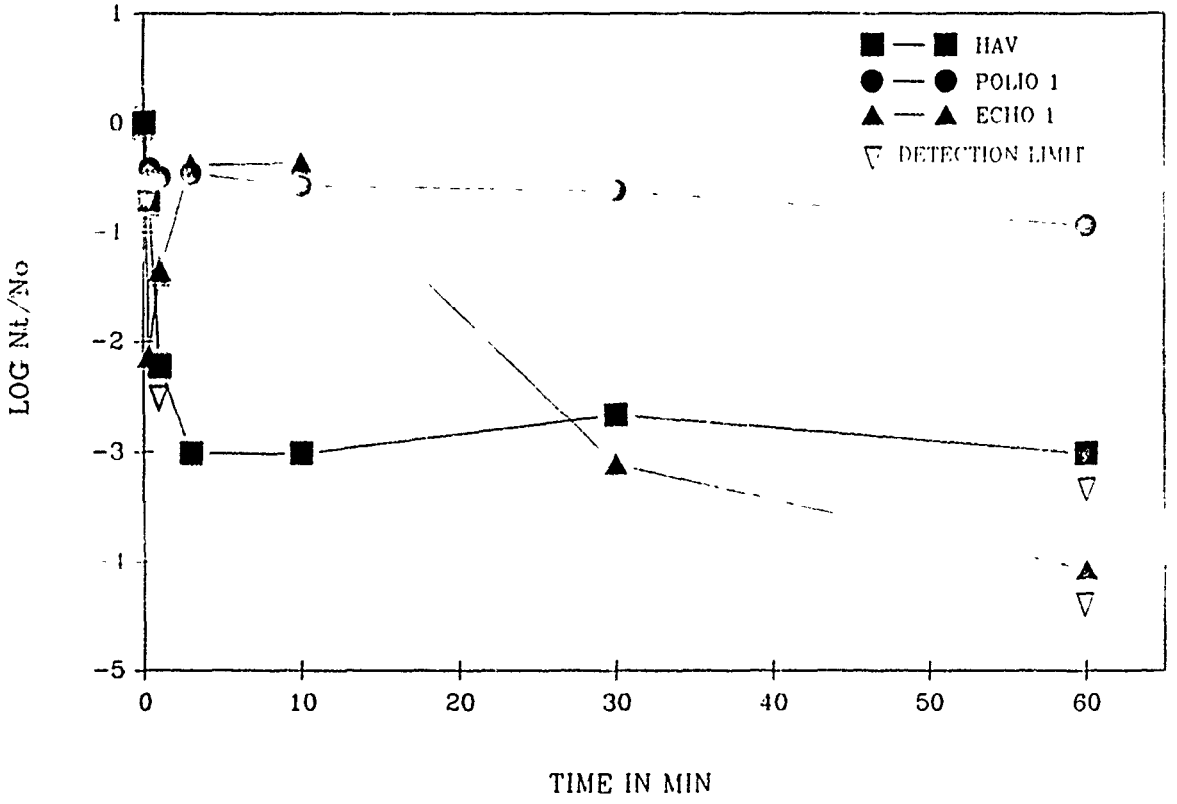


FIGURE 32. INACTIVATION OF HAV, POLIOVIRUS 1 AND ECHOVIRUS 1 BY 1 TABLET PER QUART IN WORST CASE WATER AT pH 7.0 AND 5°C.

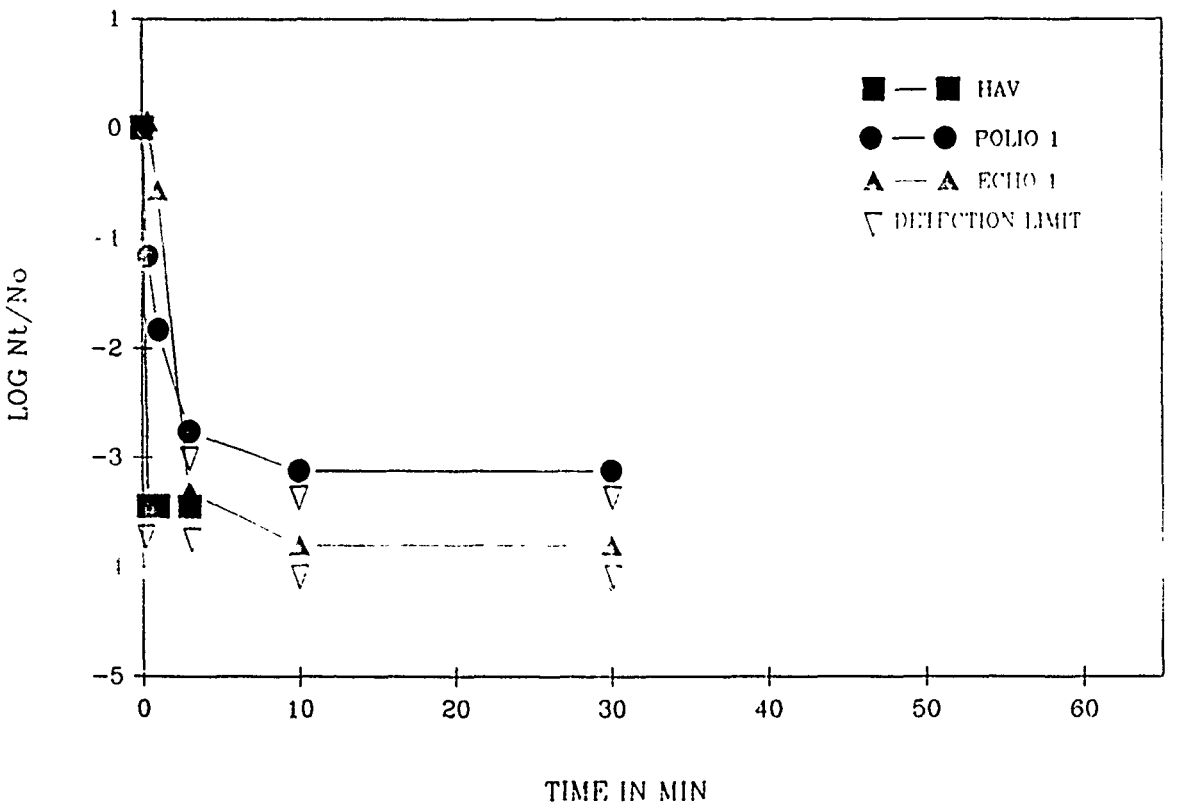


FIGURE 33. INACTIVATION OF HAV, POLIOVIRUS 1 AND ECHOVIRUS 1 BY 1 TABLET PER QUART IN WORST CASE WATER AT pH 9.5 AND 5°C.

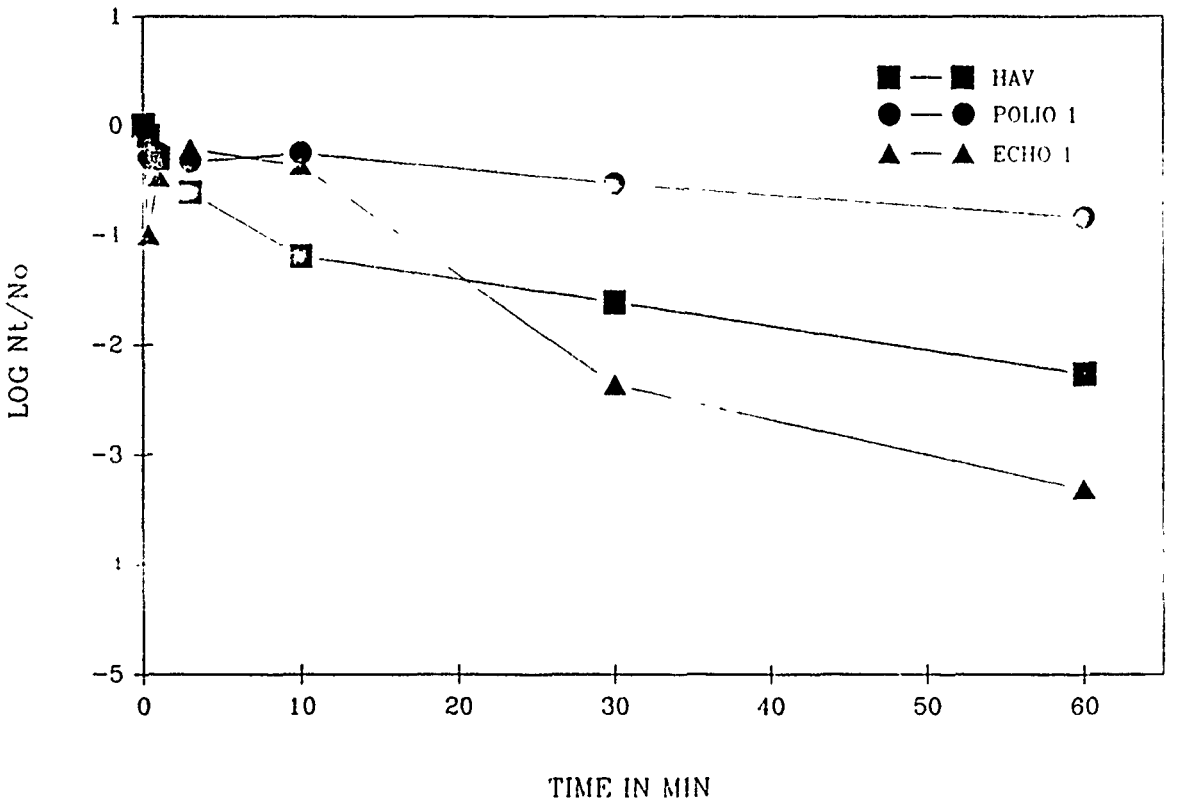


FIGURE 34. INACTIVATION OF HAV, POLIOVIRUS 1 AND ECHOVIRUS 1 BY 2 TABLETS PER QUART IN WORST CASE WATER AT pH 4.5 AND 5°C.

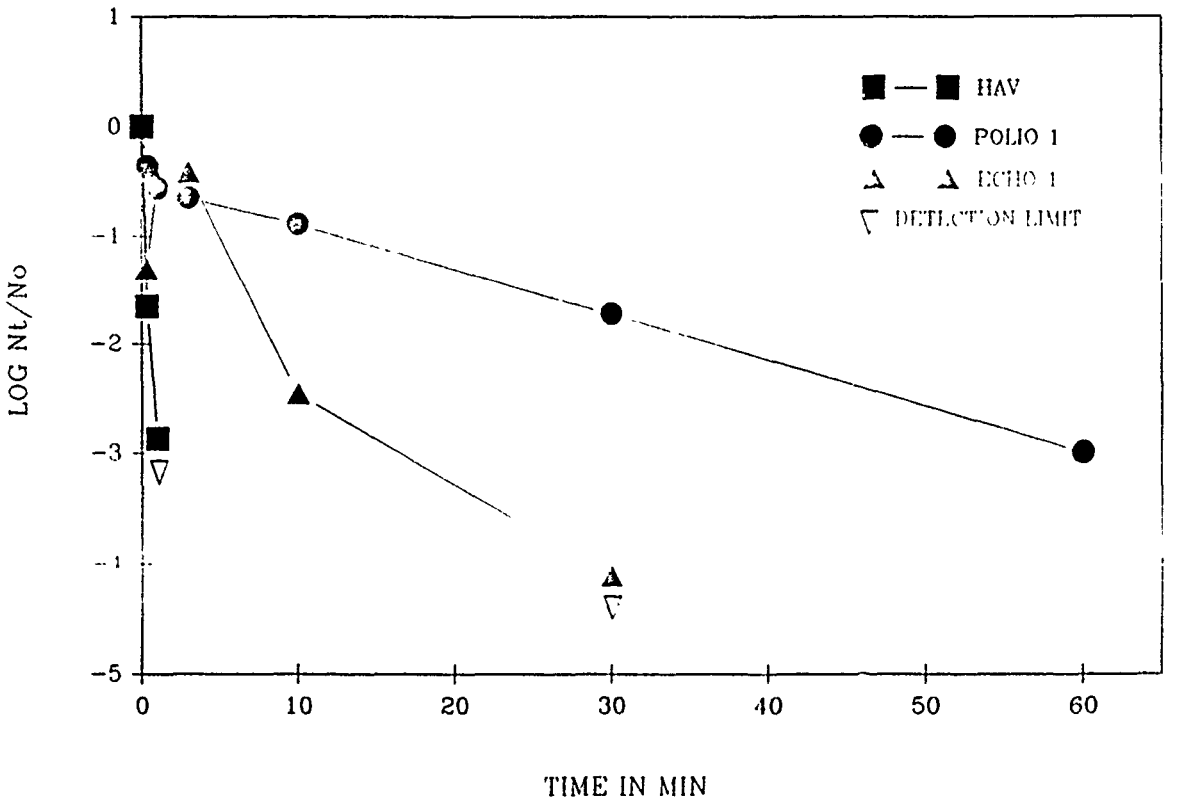


FIGURE 35. INACTIVATION OF HAV, POLIOVIRUS 1 AND ECHOVIRUS 1 BY 2 TABLETS PER QUART IN WORST CASE WATER AT pH 7.0 AND 5°C.

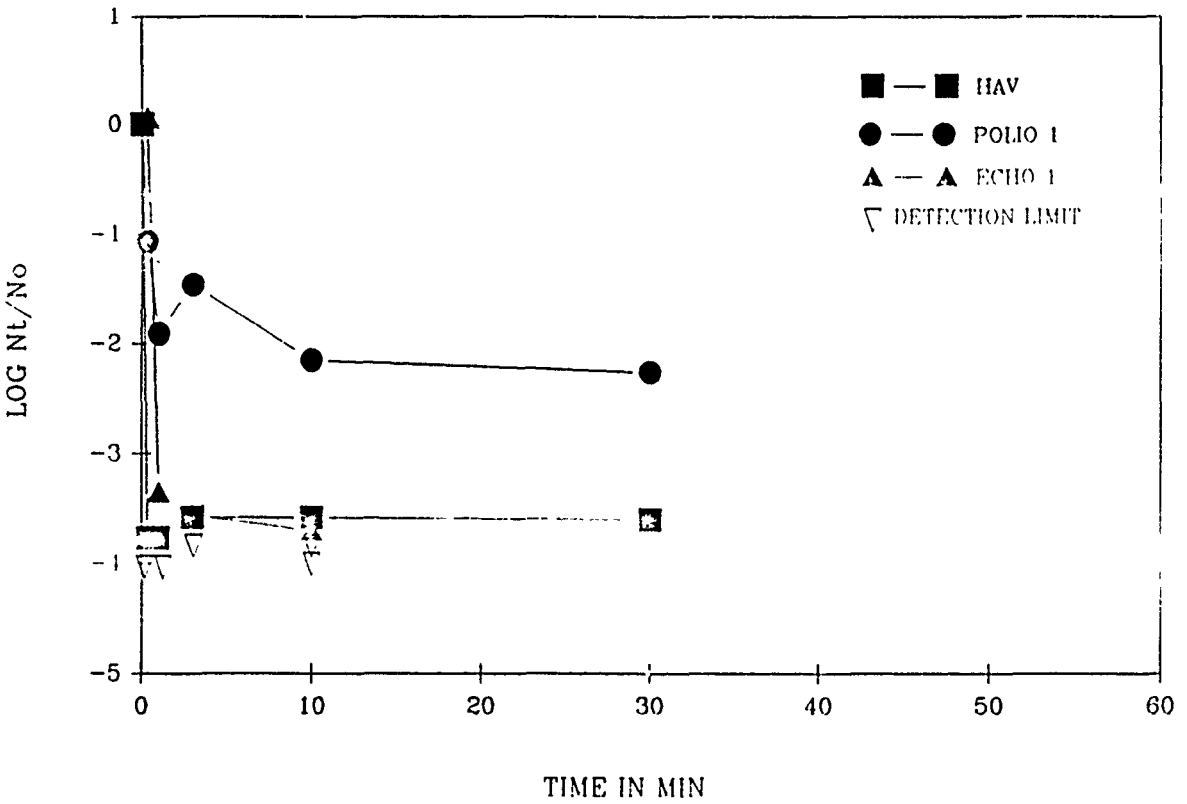


FIGURE 36. INACTIVATION OF HAV, POLIOVIRUS 1 AND ECHOVIRUS 1 BY 2 TABLETS PER QUART IN WORST CASE WATER AT pH 9.5 AND 5°C.

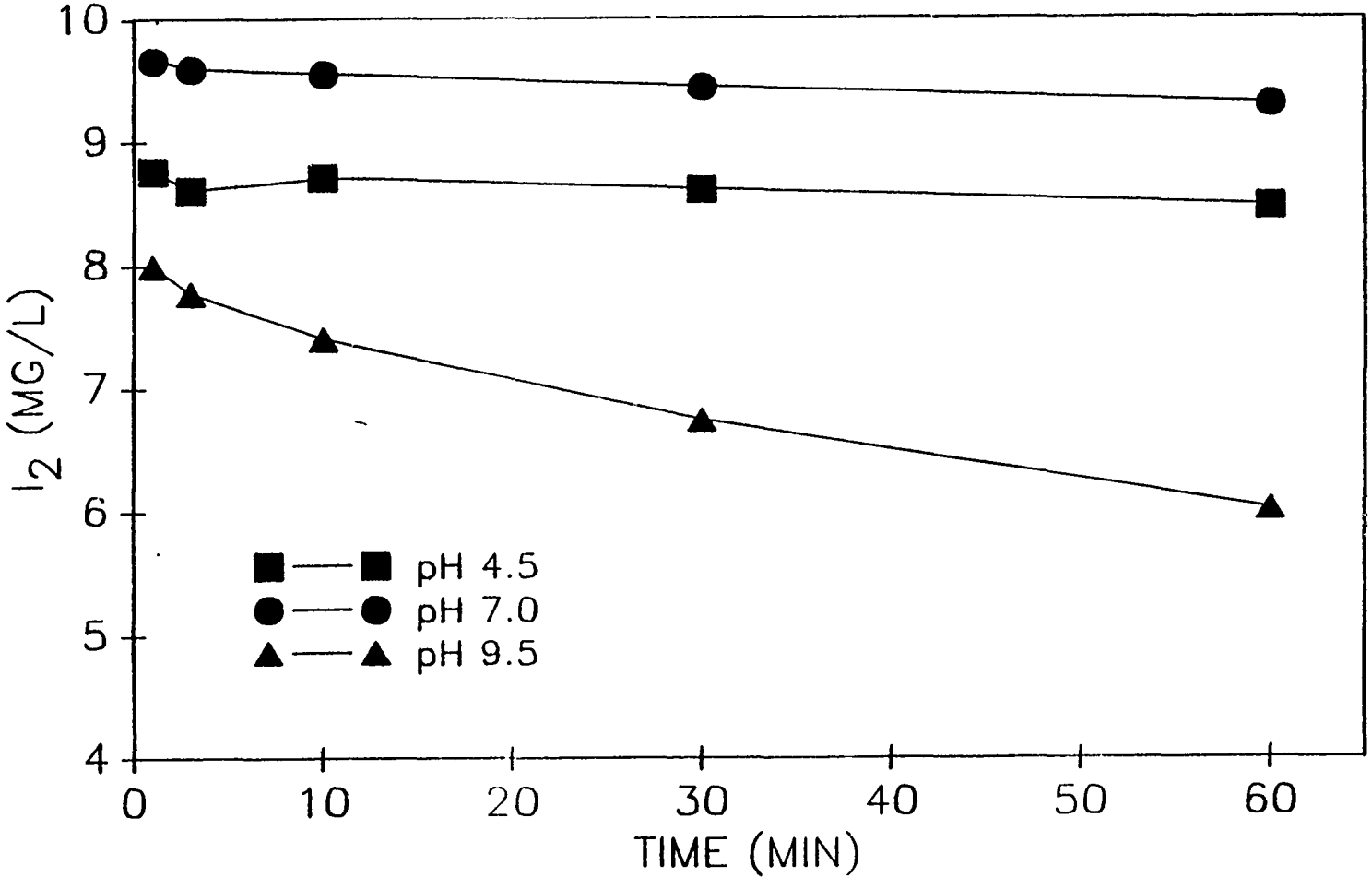


FIGURE 37. STABILITY OF 1 GLOBALINE TABLET/QUART IN PBHDFW, pH 4.5, 7.0 AND 9.5 AND 5°C.

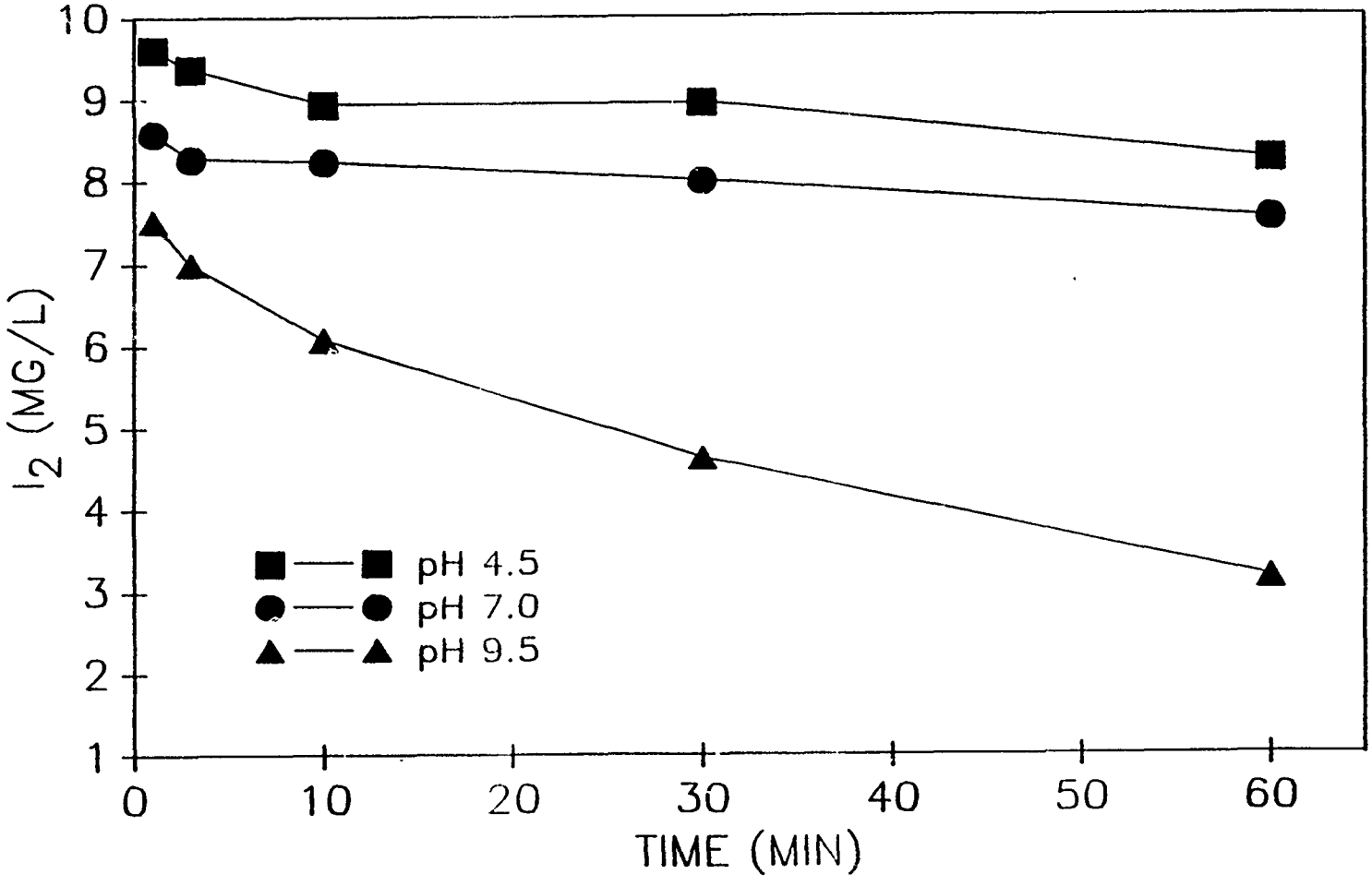


FIGURE 38. STABILITY OF 1 GLOBALINE TABLET/QUART IN PBHDFW, pH 4.5, 7.0 AND 9.5 AND 25°C.

Figure 39A. STABILITY OF 1 TABLET/QUART IODINE IN WORST CASE WATER, 5 C

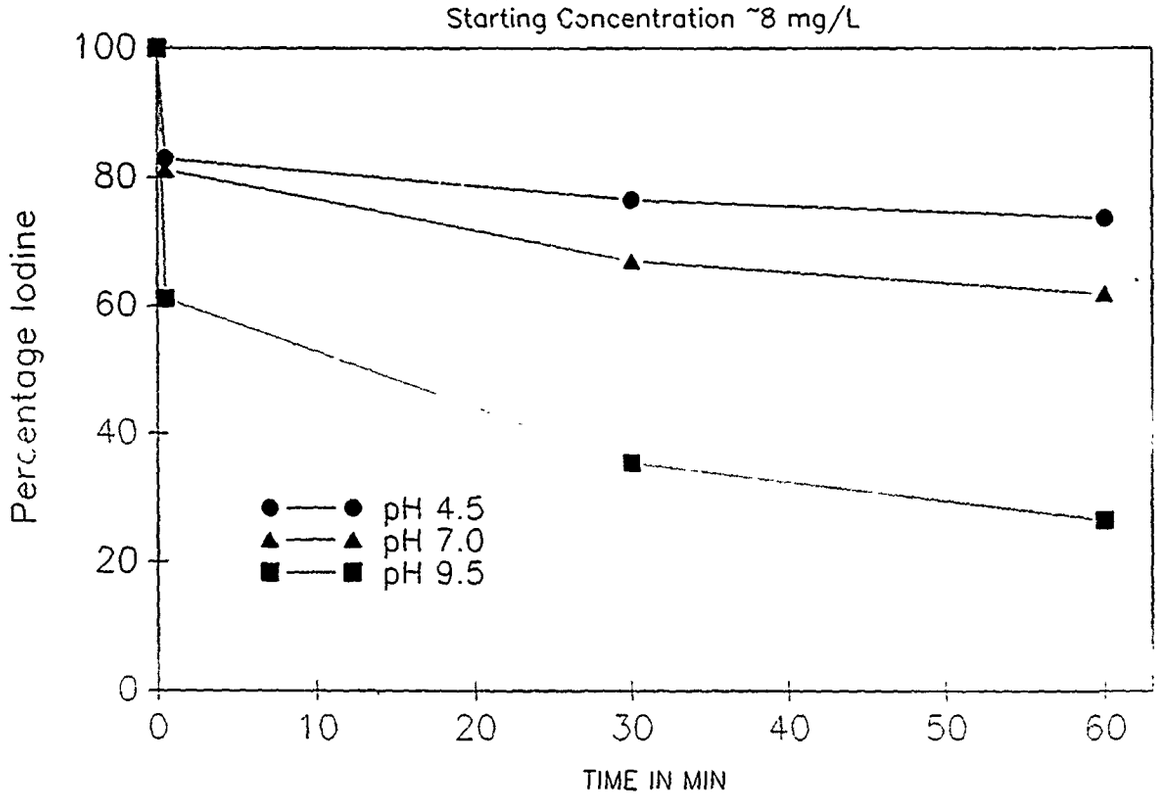


Figure 39B. STABILITY OF 2 TABLETS/QUART IODINE IN WORST CASE WATER, 5 C

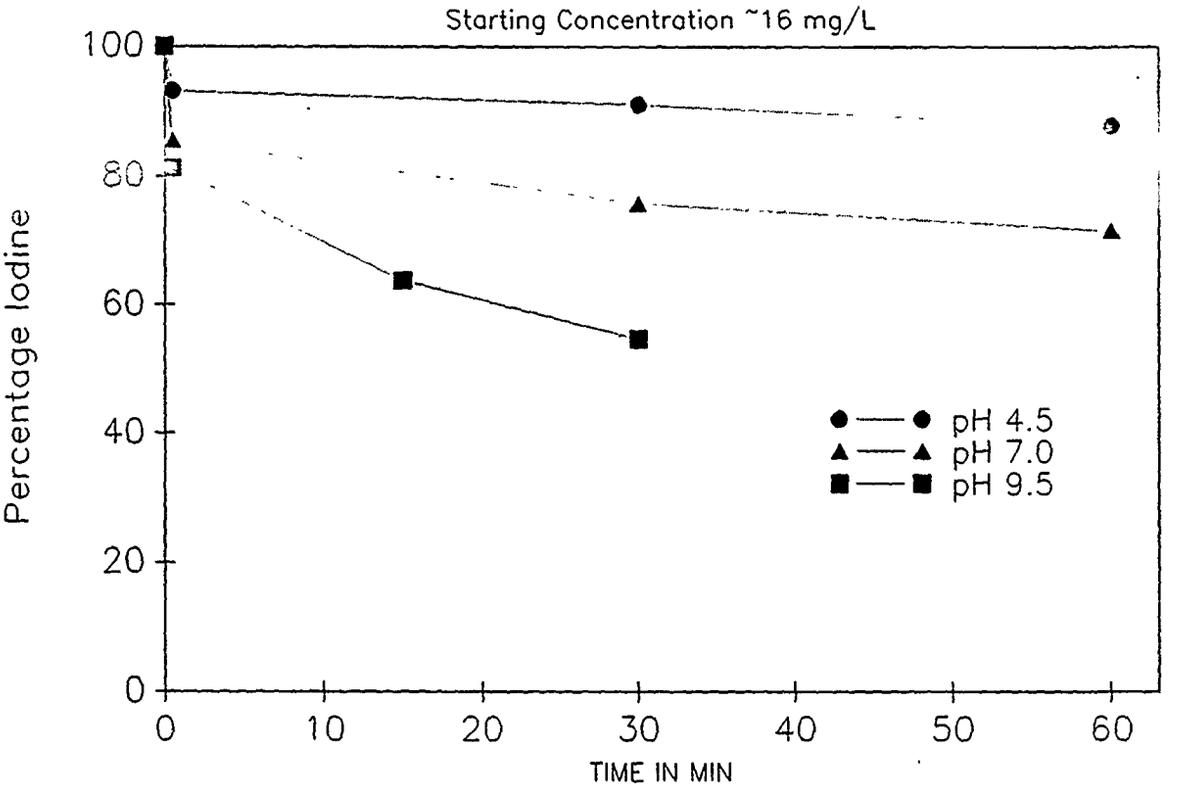


FIGURE 40A. PERCENTAGE IODINE REMAINING IN VIRUS TEST SAMPLE

Starting Concentration ~8 mg/L

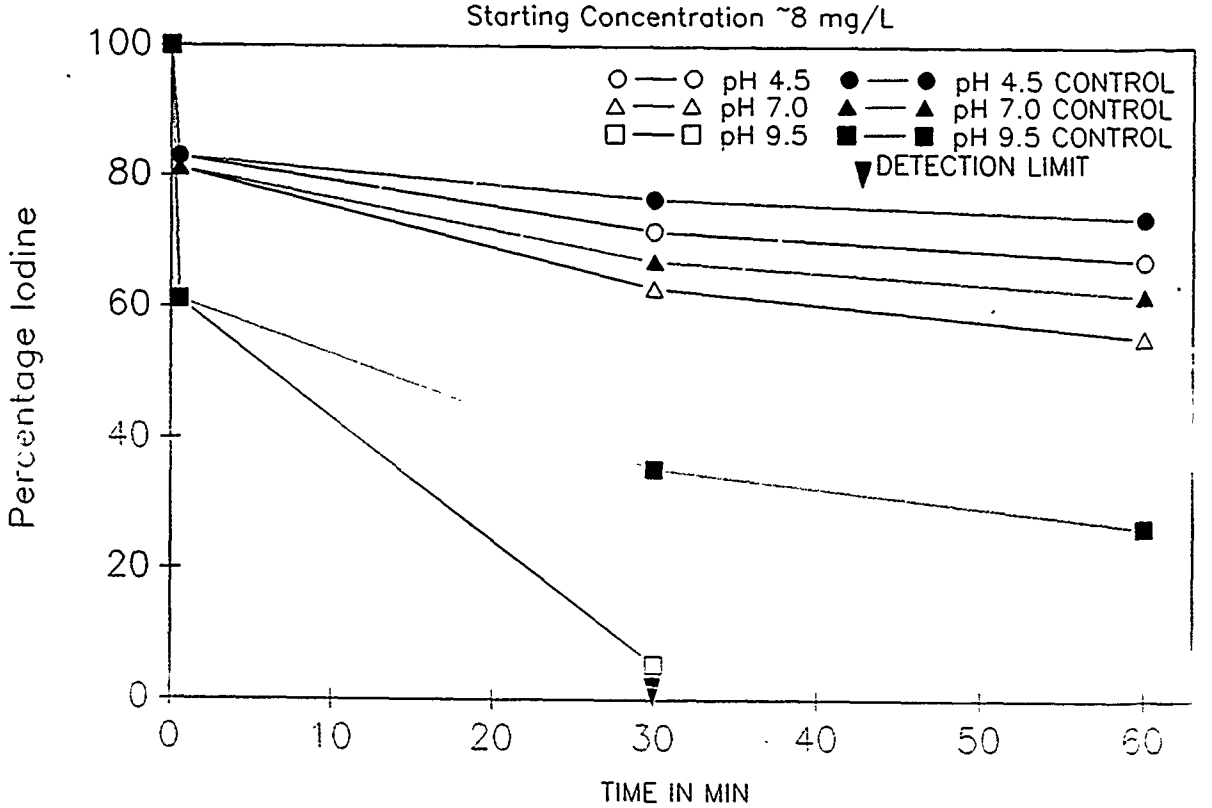
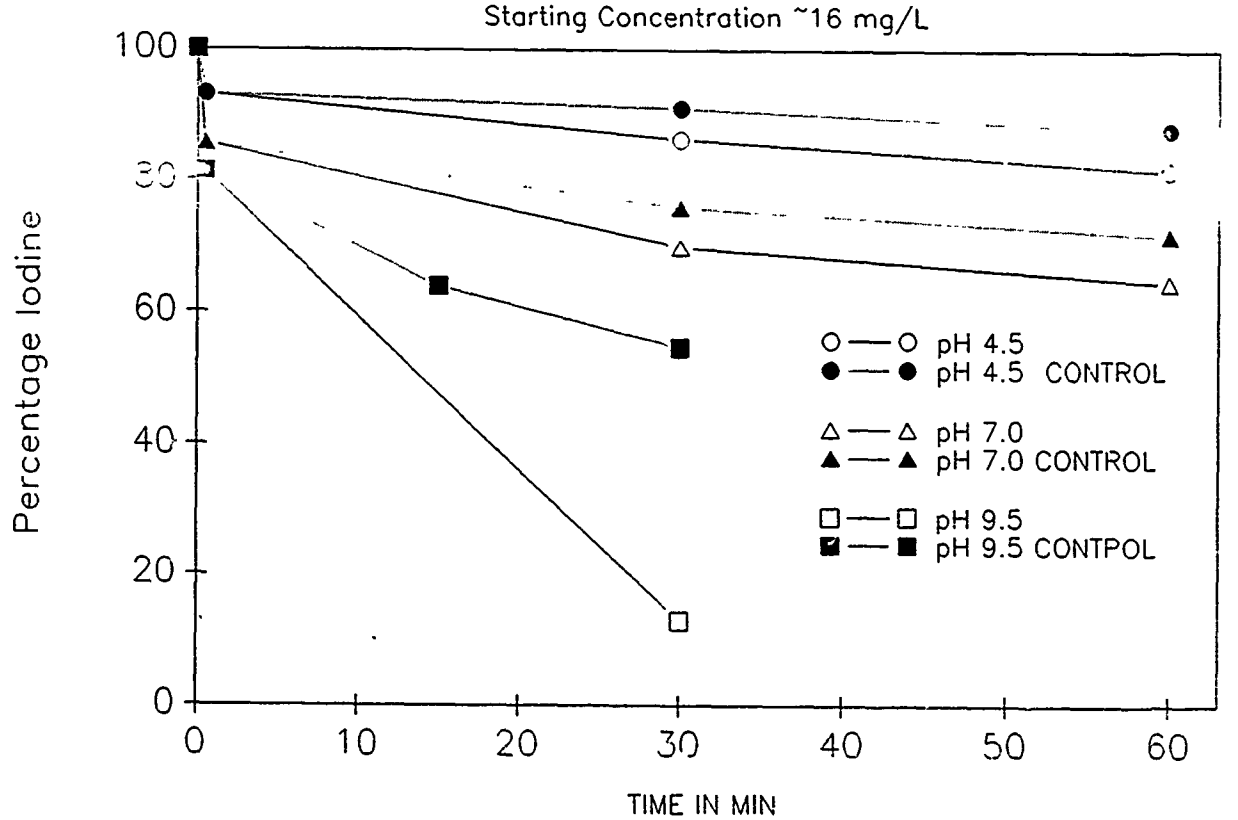


FIGURE 40B. PERCENTAGE IODINE REMAINING IN VIRUS TEST SAMPLE

Starting Concentration ~16 mg/L



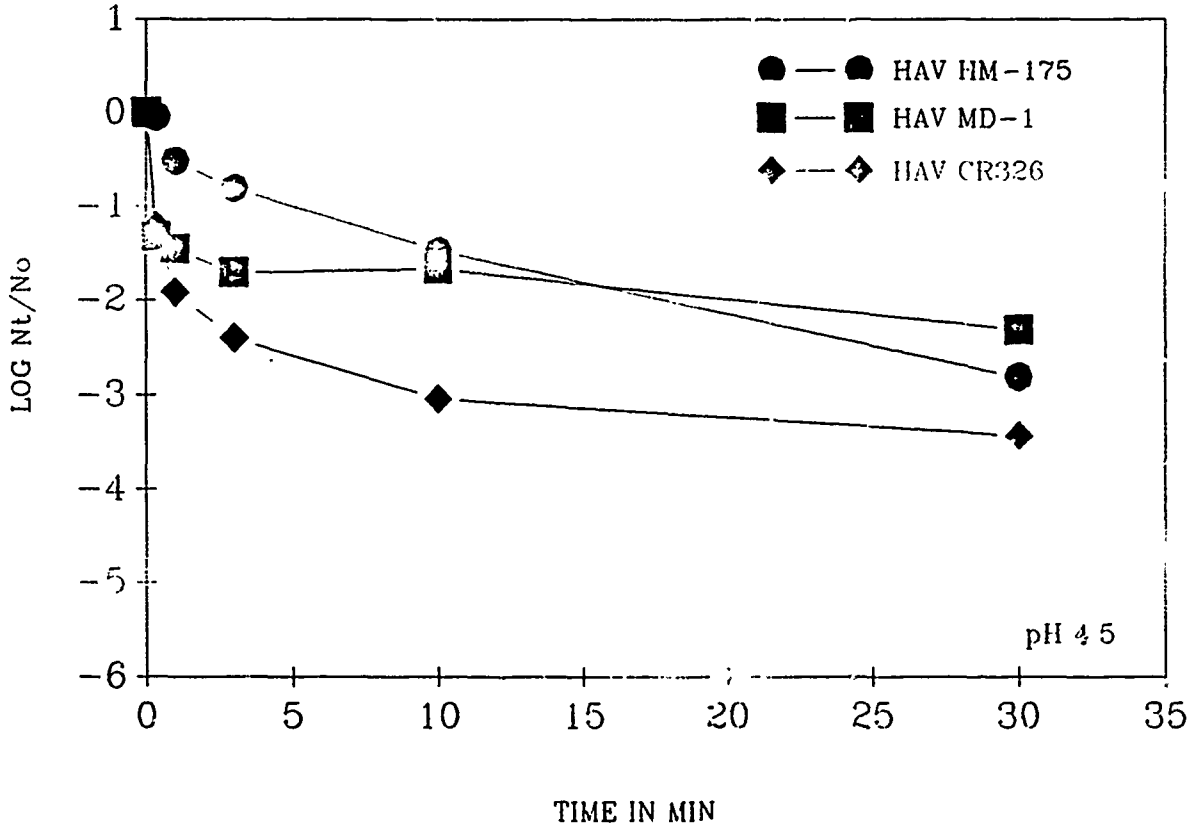


FIGURE 41. INACTIVATION OF HAV STRAINS HM175, MD-1 AND CR326 BY 1 TABLET OF IODINE PER QUART IN PBDF WATER AT pH 4.5 AND 5°C

APPENDIX A

Table 1. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 1.0 mg/L at pH 4.5 and 5C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 1.08 mg/L
60'= 0.04 mg/L

VIRUS =	HAV					
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.80E+03	5.00E-02	9.33E+03	2.63E-01	1.56E-01	-0.81
TS-1'	4.67E+03	8.34E-02	2.80E+03	7.89E-02	8.11E-02	-1.09
TS-3'	4.67E+02	8.34E-03	1.02E+02	2.87E-03	5.61E-03	-2.25
TS-10'	< 6.67E+00	< 1.19E-04	< 6.67E+00	< 1.88E-04	< 1.53E-04	> -3.81
TS-30'						
TS-60'						
AVG VC	5.60E+04		3.55E+04			
VC-0'	8.40E+04		4.11E+04			
VC-60'	2.80E+04		2.99E+04			

VIRUS=	POLIO 1					
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.68E+03	3.63E-02	1.12E+03	5.23E-02	4.43E-02	-1.35
TS-1'	8.40E+02	1.82E-02	6.53E+02	3.05E-02	2.43E-02	-1.61
TS-3'	< 6.67E+00	< 1.44E-04	6.53E+01	3.05E-03	1.60E-03	-2.80
TS-10'			< 6.67E+00	< 3.12E-04	< 1.56E-04	> -3.81
TS-30'						
TS-60'						
AVG VC	4.63E+04		2.14E+04			
VC-0'	3.74E+04		2.60E+04			
VC-60'	5.51E+04		1.68E+04			

VIRUS =	ECHO 1					
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.73E+04	5.88E-01	2.24E+04	2.82E+00	1.71E+00	0.23
TS-1'	2.71E+03	9.22E-02	1.77E+03	2.23E-01	1.58E-01	-0.80
TS-3'	8.40E+01	2.86E-03	3.73E+02	4.70E-02	2.49E-02	-1.60
TS-10'	< 6.67E+00	< 2.27E-04	< 6.67E+00	< 8.41E-04	< 5.34E-04	> -3.27
TS-30'						
TS-60'						
AVG VC	2.94E+04		7.94E+03			
VC-0'	1.77E+04		7.47E+03			
VC-60'	4.11E+04		8.40E+03			

a. < and > symbols indicate a detection limit value.

Table II. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 1.0 mg/L at pH 4.5 and 25C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 0.98mg/L
TS 60'= 0.12 mg/L

VIRUS =	HAV					
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.90E+03	2.58E-01	8.80E+03	1.98E-01	2.28E-01	-0.64
TS-1'	1.73E+03	9.11E-02	3.70E+02	8.34E-03	4.97E-02	-1.30
TS-3'	< 6.67E+00	< 3.51E-04	< 6.67E+00	< 1.50E-04	< 2.51E-04	> -3.60
TS-10'						
TS-30'						
TS-60'						
AVG VC	1.90E+04		4.44E+04			
VC-0'	3.80E+04		5.27E+04			
VC-60'	NAa		3.60E+04			

VIRUS=	POLIO 1					
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.20E+02	2.80E-02	7.00E+02	2.47E-02	2.63E-02	-1.58
TS-1'	< 6.67E+00	< 4.45E-04	< 6.67E+00	< 2.35E-04	< 3.40E-04	> -3.47
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	1.50E+04		2.84E+04			
VC-0'	3.00E+04		2.20E+04			
VC-60'	NA		3.47E+04			

VIRUS =	ECHO 1					
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.12E+03	4.37E-02	1.00E+03	1.90E-02	3.13E-02	-1.50
TS-1'	< 6.67E+00	< 2.60E-04	< 6.67E+00	< 1.27E-04	< 1.93E-04	> -3.71
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	2.57E+04		5.27E+04			
VC-0'	5.13E+04		5.20E+04			
VC-60'	NA		5.33E+04			

a. < and > symbols indicate a detection limit point.

Table III. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 1.0 mg/L at pH 7.0 and 5C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 0.98 mg/L
TS 60'= 0.32 mg/L

VIRUS = HAV						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.31E+02	5.10E-03	9.33E+02	1.67E-02	1.09E-02	-1.96
TS-1'	< 6.67E+00	< 2.60E-04	< 6.67E+00	< 1.19E-04	< 1.89E-04	> -3.72
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	2.57E+04		5.60E+04			
VC-0'	2.71E+04		6.53E+04			
VC-60'	2.43E+04		4.67E+04			
VIRUS= POLIO 1						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.76E+01	6.54E-04	1.77E+03	4.27E-02	2.17E-02	-1.66
TS-1'	2.80E+01	3.85E-04	3.72E+01	8.96E-04	6.41E-04	-3.19
TS-3'	< 6.67E+00	< 9.16E-05	< 6.67E+00	< 1.61E-04	< 1.26E-04	> -3.90
TS-10'						
TS-30'						
TS-60'						
AVG VC	7.28E+04		4.15E+04			
VC-0'	9.33E+04		4.57E+04			
VC-60'	5.23E+04		3.73E+04			
VIRUS = ECHO 1						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.24E+04	6.32E-01	7.19E+04	1.79E+00	1.21E+00	0.08
TS-1'	5.60E+02	1.58E-02	7.46E+03	1.86E-01	1.01E-01	-1.00
TS-3'	2.80E+01	7.90E-04	< 6.67E+00	< 1.66E-04	4.78E-04	-3.32
TS-10'	< 6.67E+00	< 1.88E-04			< 9.41E-05	> -4.03
TS-30'						
TS-60'						
AVG VC	3.55E+04		4.02E+04			
VC-0'	3.64E+04		2.80E+04			
VC-60'	3.45E+04		5.23E+04			

a. < and > symbols indicate a detection limit point.

Table IV. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 1.0 mg/L at pH 7.0 and 25C in halogen demand free water.a

AVG CL2 CONCENTRATION 0'= 1.0 mg/L
60'= 0.12 mg/L

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 1.61E-04	< 6.67E+00	< 5.11E-04	< 3.36E-04	> -3.47
TS-1'						
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	4.16E+04		1.31E+04			
VC-0'	3.64E+04		1.49E+04			
VC-60'	4.67E+04		1.12E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.80E+01	7.79E-04	1.68E+02	3.07E-04	5.43E-04	-3.27
TS-1'	< 6.67E+00	< 1.86E-04	< 6.67E+00	< 1.22E-05	< 9.89E-05	> -4.00
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	3.60E+04		5.48E+05			
VC-0'	5.60E+04		5.46E+05			
VC-60'	1.59E+04		5.49E+05			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.80E+02	1.26E-03	7.00E+02	2.00E-01	1.01E-01	-1.00
TS-1'	< 6.67E+00	< 3.01E-05	< 6.67E+00	< 1.91E-03	< 9.68E-04	> -3.01
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	2.22E+05		3.50E+03			
VC-0'	2.34E+04		3.36E+03			
VC-60'	4.20E+05		3.64E+03			

a. < and > symbols indicate a detection limit point.

Table V. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 1.0 mg/L at pH 9.5 and 5C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 1.1 mg/L
60'= 0.54 mg/L

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.21E+04	2.49E-01	2.89E+04	9.25E-01	5.87E-01	-0.23
TS-1'	1.87E+03	3.85E-02	1.87E+04	5.98E-01	3.18E-01	-0.50
TS-3'	3.73E+02	7.68E-03	1.21E+03	3.87E-02	2.32E-02	-1.63
TS-10'	< 6.67E+00	< 1.37E-04	< 6.67E+00	< 2.13E-04	< 1.75E-04	> -3.76
TS-30						
TS-60'						
AVG VC	4.86E+04		3.13E+04			
VC-0'	3.73E+04		3.64E+04			
VC-60'	5.98E+04		2.61E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.68E+04	8.00E-01	2.33E+04	9.79E-01	8.89E-01	-0.05
TS-1'	1.96E+04	9.33E-01	1.59E+04	6.68E-01	8.01E-01	-0.10
TS-3'	1.03E+04	4.90E-01	1.31E+04	5.50E-01	5.20E-01	-0.28
TS-10'	1.59E+04	7.57E-01	5.64E+03	2.37E-01	4.97E-01	-0.30
TS-30'	3.73E+01	1.78E-03	2.61E+02	1.10E-02	6.37E-03	-2.20
TS-60'	< 6.67E+00	< 3.18E-04	< 6.67E+00	< 2.80E-04	< 2.99E-04	> -3.52
AVG VC	2.10E+04		2.38E+04			
VC-0'	2.05E+04		1.96E+04			
VC-60'	2.15E+04		2.80E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.12E+05	1.71E+00	1.87E+04	7.15E-01	1.21E+00	0.08
TS-1'	1.12E+05	1.71E+00	1.40E+05	5.35E+00	3.53E+00	0.55
TS-3'	1.87E+05	2.86E+00	2.15E+05	8.22E+00	5.54E+00	0.74
TS-10'	NA	NA	1.60E+05	6.12E+00	3.06E+00	0.49
TS-30'	1.86E+04	2.85E-01	1.03E+05	3.94E+00	2.11E+00	0.32
TS-60'	1.12E+04	1.71E-01	5.60E+04	2.14E+00	1.16E+00	0.06
AVG VC	6.54E+04		2.62E+04			
VC-0'	7.47E+04		9.33E+03			
VC-60'	5.60E+04		4.30E+04			

a. < and > symbols indicate a detection limit point.

Table VI. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 1.0 mg/L at pH 9.5 and 25C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 1.0 mg/L
60'= 0.34 mg/L

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.68E+04	2.61E-01	1.18E+04	1.77E-01	2.19E-01	-0.66
TS-1'	8.07E+02	1.26E-02	1.20E+03	1.80E-02	1.53E-02	-1.82
TS-3'	< 6.67E+00	< 1.04E-04	< 6.67E+00	< 1.00E-04	< 1.02E-04	> -3.99
TS-10'						
TS-30'						
TS-60'						
AVG VC	6.43E+04		6.67E+04			
VC-0'	7.33E+04		6.67E+04			
VC-60'	5.53E+04		6.67E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.70E+04	4.91E-01	3.10E+04	5.23E-01	5.07E-01	-0.30
TS-1'	1.94E+04	3.53E-01	2.05E+04	3.46E-01	3.49E-01	-0.46
TS-3'	8.20E+02	1.49E-02	3.27E+03	5.51E-02	3.50E-02	-1.46
TS-10'	< 6.67E+00	< 1.21E-04	< 6.67E+00	< 1.12E-04	< 1.17E-04	> -3.93
TS-30'						
TS-60'						
AVG VC	5.50E+04		5.93E+04			
VC-0'	5.53E+04		6.33E+04			
VC-60'	5.47E+04		5.53E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.13E+04	8.26E-01	4.93E+04	1.66E+00	1.24E+00	0.09
TS-1'	6.90E+04	1.38E+00	9.73E+04	3.28E+00	2.33E+00	0.37
TS-3'	2.67E+03	5.34E-02	7.53E+04	2.54E+00	1.30E+00	0.11
TS-10'	5.00E+03	1.00E-01	2.04E+03	6.88E-02	8.44E-02	-1.07
TS-30'	< 6.67E+00	< 1.33E-04	< 6.67E+00	< 2.25E-04	< 1.79E-04	> -3.75
TS-60'						
AVG VC	5.00E+04		2.97E+04			
VC-0'	4.87E+04		3.53E+04			
VC-60'	5.13E+04		2.40E+04			

a. < and > symbols indicate a detection limit point.

Table VII. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 5 mg/L at pH 4.5 and 5C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 4.97 mg/L
60'= 4.1 mg/L

VIRUS = HAV						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	5.87E+03	2.24E-02	1.04E+04	4.45E-02	3.35E-02	-1.48
TS-1'	6.67E+01	2.55E-04	4.67E+02	2.00E-03	1.13E-03	-2.95
TS-3'	< 6.67E+00	< 2.55E-05	< 6.67E+00	< 2.86E-05	< 2.70E-05	> -4.57
TS-10'						
TS-30'						
TS-60'						
AVG VC	2.62E+05		2.34E+05			
VC-0'	2.80E+05		2.67E+05			
VC-60'	2.44E+05		2.00E+05			

VIRUS= POLIO 1						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.33E+01	6.54E-04	1.13E+02	7.22E-03	3.94E-03	-2.40
TS-1'	< 6.67E+00	< 3.28E-04	< 6.67E+00	< 4.26E-04	< 3.77E-04	> -3.42
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	2.04E+04		1.57E+04			
VC-0'	2.00E+04		1.80E+04			
VC-60'	2.07E+04		1.33E+04			

VIRUS = ECHO 1						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 1.28E-04	6.00E+01	1.21E-03	6.68E-04	-3.18
TS-1'	0.00E+00	0.00E+00	< 6.67E+00	< 1.34E-04	< 6.71E-05	> -4.17
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	5.20E+04		4.97E+04			
VC-0'	5.07E+04		5.07E+04			
VC-60'	5.33E+04		4.87E+04			

a. < and > symbols indicate a detection limit point.

Table VIII. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 5.0 mg/L at pH 4.5 and 25C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 5.2 mg/L
30'= 5.0 mg/L

VIRUS = HAV						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.00E+01	1.59E-03	4.33E+02	6.56E-03	4.07E-03	-2.39
TS-1'	< 6.67E+00	< 5.29E-04	< 6.67E+00	< 1.01E-04	< 3.15E-04	> -3.50
TS-3'						
TS-10'						
TS-30'						
AVG VC	1.26E+04		6.60E+04			
VC-0'	1.20E+04		8.13E+04			
VC-30'	1.32E+04		5.07E+04			

VIRUS= POLIO 1						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 7.52E-04	< 6.67E+00	< 2.41E-04	< 4.97E-04	> -3.30
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	8.87E+03		2.77E+04			
VC-0'	9.00E+03		3.27E+04			
VC-30'	8.73E+03		2.27E+04			

VIRUS = ECHO 1						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 7.88E-04	< 6.67E+00	< 1.69E-03	< 1.24E-03	> -2.91
TS-1'						
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	8.47E+03		3.95E+03			
VC-0'	8.13E+03		3.90E+03			
VC-60'	8.80E+03		4.00E+03			

a. < and > symbols indicate a detection limit point.

Table IX. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 5.0 mg/L at pH 7.0 and 5C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 5.1 mg/L
60'= 3.3 mg/L

VIRUS = HAV						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	3.40E+03	2.17E-02	5.00E+02	4.83E-03	1.32E-02	-1.88
TS-1'	2.67E+01	1.70E-04	< 6.67E+00	< 6.44E-05	1.17E-04	-3.93
TS-3'	< 6.67E+00	< 4.25E-05			< 2.12E-05	> -4.67
TS-10'						
TS-30'						
TS-60'						
AVG VC	1.57E+05		1.04E+05			
VC-0'	1.47E+05		1.07E+05			
VC-60'	1.67E+05		1.00E+05			

VIRUS= POLIO 1						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	8.73E+02	2.26E-02	2.60E+01	8.48E-04	1.17E-02	-1.93
TS-1'	2.00E+01	5.17E-04	< 6.67E+00	< 2.18E-04	3.68E-04	-3.43
TS-3'	< 6.67E+00	< 1.73E-04			< 8.63E-05	> -4.06
TS-10'						
TS-30'						
TS-60'						
AVG VC	3.87E+04		3.07E+04			
VC-0'	4.60E+04		2.53E+04			
VC-60'	3.13E+04		3.60E+04			

VIRUS = ECHO 1						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.33E+02	9.77E-03	< 6.67E+00	< 1.35E-04	4.95E-03	-2.30
TS-1'	< 6.67E+00	< 1.51E-04			< 7.53E-05	> -4.12
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	4.43E+04		4.94E+04			
VC-0'	4.73E+04		4.80E+04			
VC-60'	4.13E+04		5.07E+04			

a. < and > indicates a detection limit point.

Table X. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 5.0 mg/L at pH 7.0 and 25C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 5.05 mg/L
60'= 4.6 mg/L

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 2.50E-03	< 6.67E+00	< 1.28E-04	< 1.31E-03	> -2.88
TS-1'						
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	2.67E+03		5.23E+04			
VC-0'	2.67E+03		5.73E+04			
VC-60'	2.67E+03		4.73E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 1.26E-03	< 6.67E+00	< 2.57E-04	< 7.58E-04	> -3.12
TS-1'						
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	5.30E+03		2.60E+04			
VC-0'	5.30E+03		3.00E+04			
VC-60'	5.30E+03		2.20E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 6.20E-04	< 6.67E+00	< 1.39E-03	> 1.01E-03	-3.00
TS-1'						
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	1.08E+04		4.80E+03			
VC-0'	1.10E+04		4.80E+03			
VC-60'	1.05E+04		4.80E+03			

a. < and > symbols indicate a detection limit point.

Table XI. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 5.0 mg/L at pH 9.5 and 5C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 5.08 mg/L
60'= 2.63 mg/L

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	7.47E+03	4.76E-02	2.13E+03	1.28E-02	3.02E-02	-1.52
TS-1'	4.73E+03	3.01E-02	2.67E+01	1.60E-04	1.51E-02	-1.82
TS-3'	< 6.67E+00	< 4.25E-05	< 6.67E+00	< 4.01E-05	< 4.13E-05	> -4.38
TS-10'						
TS-30'						
TS-60'						
AVG VC	1.57E+05		1.67E+05			
VC-0'	1.67E+05		1.60E+05			
VC-60'	1.47E+05		1.73E+05			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	5.87E+02	4.77E-02	2.00E+03	1.39E-01	9.35E-02	-1.03
TS-1'	3.47E+02	2.82E-02	1.87E+02	1.30E-02	2.06E-02	-1.69
TS-3'	1.20E+02	9.74E-03	< 6.67E+00	< 4.65E-04	5.10E-03	-2.29
TS-10'	< 6.67E+00	< 5.42E-04			< 2.71E-04	> -3.57
TS-30						
TS-60'						
AVG VC	1.23E+04		1.44E+04			
VC-0'	1.53E+04		1.80E+04			
VC-60'	9.33E+03		1.07E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	5.80E+03	1.80E-01	5.50E+03	1.55E-01	1.67E-01	-0.78
TS-1'	5.80E+03	1.80E-01	4.70E+03	1.32E-01	1.56E-01	-0.81
TS-3'	4.60E+03	1.42E-01	4.13E+03	1.16E-01	1.29E-01	-0.89
TS-10'	1.13E+03	3.50E-02	1.07E+03	3.01E-02	3.26E-02	-1.49
TS-30'	< 6.67E+00	< 2.07E-04	< 6.67E+00	< 1.88E-04	< 1.97E-04	> -3.71
TS-60'						
AVG VC	3.23E+04		3.55E+04			
VC-0'	3.33E+04		3.30E+04			
VC-60'	3.13E+04		3.80E+04			

a. < and > symbols indicate a detection limit point.

Table XII. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 5.0 mg/L at pH 9.5 and 25C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 5.08 mg/L
60'= 4.8 mg/L

VIRUS = HAV						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.90E+02	9.05E-03	2.20E+02	3.79E-03	6.42E-03	-2.19
TS-1'	6.67E+00	3.18E-04	< 6.67E+00	< 1.15E-04	< 2.16E-04	> -3.66
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	2.10E+04		5.80E+04			
VC-0'	2.13E+04		5.30E+04			
VC-60'	2.07E+04		6.30E+04			

VIRUS= POLIO 1						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	6.07E+02	3.64E-01	4.20E+03	1.60E-01	2.62E-01	-0.58
TS-1'	7.30E+01	4.37E-02	3.33E+01	1.27E-03	2.25E-02	-1.65
TS-3'	< 6.67E+00	< 4.00E-03	< 6.67E+00	< 2.54E-04	< 2.12E-03	> -2.67
TS-10'						
TS-30'						
TS-60'						
AVG VC	1.67E+03		2.63E+04			
VC-0'	2.67E+03		3.33E+04			
VC-60'	6.67E+02		1.93E+04			

VIRUS = ECHO 1						
SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.90E+04	4.43E+00	1.47E+04	3.37E+00	3.90E+00	0.59
TS-1'	2.90E+04	2.62E+00	4.27E+03	9.78E-01	1.80E+00	0.26
TS-3'	2.80E+03	2.53E-01	< 6.67E+00	< 1.53E-03	1.27E-01	-0.89
TS-10'	< 6.67E+00	< 6.04E-04			< 3.02E-04	> -3.52
TS-30'						
TS-60'						
AVG VC	1.11E+04		4.37E+03			
VC-0'	1.01E+04		4.60E+03			
VC-60'	1.20E+04		4.13E+03			

a. < and > symbols indicate a detection limit point.

Table XIII. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 3.0 mg/L at pH 4.5 and 5C in worst case water.a

AVG Cl2 CONCENTRATION 0'= 3.33 mg/L AVG HUMIC-FULVIC CONC = 9.89 mg/L
30'= 1.95 mg/L AVG BENTONITE CLAY CONC = 5.1 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	8.70E+01	1.60E-02	1.13E+01	2.11E-03	9.04E-03	-2.04
TS-1'	2.00E+01	3.67E-03	< 6.67E+00	< 1.25E-03	2.46E-03	-2.61
TS-3'	< 6.67E+00	< 1.22E-03			< 6.12E-04 >	-3.21
TS-10'						
TS-30'						
AVG VC-WC	5.45E+03		5.35E+03			
VC-WC 0'	4.70E+03		6.00E+03	VC-HDF 0'	ND b	ND
VC-WC 30'	6.20E+03		4.70E+03	VC-HDF 30'	ND	ND

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.00E+03	1.18E-01	9.87E+02	5.77E-02	8.79E-02	-1.06
TS-1'	1.30E+01	7.67E-04	< 6.67E+00	< 3.90E-04	5.79E-04	-3.24
TS-3'	< 6.67E+00	< 3.94E-04			< 1.97E-04 >	-3.71
TS-10'						
TS-30'						
AVG VC	1.70E+04		1.71E+04			
VC-WC 0'	1.81E+04		1.81E+04	VC-HDF 0'	9.00E+03	9.90E+03
VC-WC 30'	1.58E+04		1.61E+04	VC-HDF 30'	6.90E+03	2.13E+03

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	8.20E+03	8.12E-01	1.13E+01	1.14E-03	4.07E-01	-0.39
TS-1'	< 6.67E+00	< 6.60E-04	< 6.67E+00	< 6.75E-04	< 6.68E-04 >	-3.18
TS-3'						
TS-10'						
TS-30'						
AVG VC-WC	1.01E+04		9.89E+03			
VC-0'	1.05E+04		1.11E+04	VC-HDF 0'	1.20E+04	7.73E+03
VC-30'	9.70E+03		8.67E+03	VC-HDF 30'	6.67E+03	1.73E+03

a. < and > symbols indicate a detection limit point.
b. Not done.

Table XIV. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 3.0 mg/L free chlorine at pH 4.5 and 25C in worst case water.a

AVG Cl2 CONCENTRATION 0'= 3.01 mg/L AVG HUMIC-FULVIC ACID CONC = 10.2 mg/L
30'= 1.65 mg/L AVG BENTONITE CLAY CONC = 5.0 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.00E+01	2.25E-03	1.33E+01	1.49E-03	1.87E-03	-2.73
TS-1'	6.67E+00	7.49E-04	< 6.67E+00	< 7.45E-04	< 7.47E-04	> -3.13
TS-3'						
TS-10'						
TS-30'						
AVG VC	8.90E+03		8.95E+03			
VC-0'	9.50E+03		8.30E+03			
VC-30'	8.30E+03		9.60E+03			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.00E+02	6.90E-03	8.70E+01	6.59E-03	6.74E-03	-2.17
TS-1'	< 6.67E+00	< 4.60E-04	6.67E+00	5.05E-04	< 4.83E-04	> -3.32
TS-3'						
TS-10'						
TS-30'						
AVG VC	1.45E+04		1.32E+04			
VC-0'	1.60E+04		1.50E+04			
VC-30'	1.30E+04		1.14E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	6.67E+00	1.01E-03	< 6.67E+00	< 9.53E-04	< 9.82E-04	> -3.01
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	6.60E+03		7.00E+03			
VC-0'	6.70E+03		6.70E+03			
VC-30'	6.50E+03		7.30E+03			

a. < and > indicates a detection limit point.

Table XV. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 3.0 mg/L at pH 7.0 and 5C in worst case water.a

AVG Cl2 CONCENTRATION 0'= 3.03 mg/L AVG HUMIC-FULVIC ACID CONC =10.4 mg/L
30'= 1.65 mg/L AVG BENTONITE CLAY CONC = 5.2 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 8.95E-04	< 6.67E+00	< 6.84E-04	< 7.90E-04	> -3.10
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC-WC	7.45E+03		9.75E+03			
VC-WC 0'	6.30E+03		1.10E+04	VC-HDF 0'	ND b	ND
VC-WC 30'	8.60E+03		8.50E+03	VC-HDF 30'	ND	ND

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.80E+02	6.27E-03	4.00E+02	8.57E-03	7.42E-03	-2.13
TS-1'	< 6.67E+00	< 1.49E-04	< 6.67E+00	< 1.43E-04	< 1.46E-04	> -3.84
TS-3'						
TS-10'						
TS-30'						
AVG VC-WC	4.47E+04		4.67E+04			
VC-WC 0'	4.73E+04		4.73E+04	VC-HDF 0'	2.30E+04	2.46E+04
VC-WC 30'	4.20E+04		4.60E+04	VC-HDF 30'	3.06E+04	3.00E+04

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.73E+03	1.37E-01	4.93E+03	2.10E-01	1.73E-01	-0.76
TS-1'	< 6.67E+00	< 1.93E-04	< 6.67E+00	< 2.84E-04	< 2.39E-04	> -3.62
TS-3'						
TS-10'						
TS-30'						
AVG VC-WC	3.45E+04		2.35E+04			
VC-WC 0'	3.30E+04		2.30E+04	VC-HDF 0'	2.50E+04	3.20E+04
VC-WC 30'	3.60E+04		2.40E+04		2.60E+04	2.60E+04

a. < and > indicates a detection limit point.
b. Not done.

Table XVI. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 3.0 mg/L at pH 7.0 and 25C in worst case water.a

AVG Cl2 CONCENTRATION 0'= 2.7 mg/L AVG HUMIC-FULVIC ACID CONC = 10.3 mg/L
30'= 0.96 mg/L AVG BENTONITE CLAY CONC = 5.1 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 1.03E-03	< 6.67E+00	< 6.74E-04	< 8.50E-04	> -3.07
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	6.50E+03		9.90E+03			
VC-0'	5.50E+03		1.02E+04			
VC-30'	7.50E+03		9.60E+03			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 3.92E-04	< 6.67E+00	< 4.17E-04	< 4.05E-04	> -3.39
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	1.70E+04		1.60E+04			
VC-0'	1.70E+04		1.30E+04			
VC-30'	1.70E+04		1.90E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 8.23E-04	1.30E+01	1.88E-03	1.35E-03	-2.87
TS-1'	0.00E+00	0.00E+00	< 6.67E+00	< 9.62E-04	< 4.81E-04	> -3.32
TS-3'						
TS-10'						
TS-30'						
AVG VC	8.10E+03		6.93E+03			
VC-0'	8.30E+03		6.73E+03			
VC-30'	7.90E+03		7.13E+03			

a. < and > indicates a detection limit point.

Table XVII. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 3.0 mg/L at pH 9.5 and 5C in worst case water.a

AVG Cl2 CONCENTRATION 0'= 3.1 mg/L AVG HUMIC-FULVIC ACID CONC = 10.4 mg/L
30'= 1.0 mg/L AVG BENTONITE CLAY CONC = 5.1 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.00E+01	1.94E-03	6.67E+00	7.54E-04	1.35E-03	-2.87
TS-1'	< 6.67E+00	< 6.48E-04			< 3.24E-04	> -3.49
TS-3'						
TS-10'						
TS-30'						
AVG VC	1.03E+04		8.85E+03			
VC-0'	1.04E+04		9.90E+03			
VC-30'	1.02E+04		7.80E+03			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	5.10E+03	4.45E-01	6.60E+03	5.06E-01	4.76E-01	-0.32
TS-1'	1.30E+03	1.14E-01	8.30E+02	6.36E-02	8.86E-02	-1.05
TS-3'	4.70E+01	4.10E-03	1.33E+01	1.02E-03	2.56E-03	-2.59
TS-10'	< 6.67E+00	< 5.83E-04	< 6.67E+00	< 5.11E-04	< 5.47E-04	> -3.26
TS-30'						
AVG VC	1.15E+04		1.31E+04			
VC-0'	1.30E+04		1.40E+04			
VC-30'	9.90E+03		1.21E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.20E+04	1.89E+00	1.50E+04	1.23E+00	1.56E+00	0.19
TS-1'	2.54E+04	2.18E+00	2.60E+04	2.13E+00	2.16E+00	0.33
TS-3'	2.83E+04	2.43E+00	2.35E+04	1.93E+00	2.18E+00	0.34
TS-10'	4.20E+03	3.61E-01	4.72E+03	3.87E-01	3.74E-01	-0.43
TS-30'	< 6.67E+00	< 5.73E-04	< 6.67E+00	< 5.47E-04	< 5.60E-04	> -3.25
AVG VC	1.17E+04		1.22E+04			
VC-0'	1.20E+04		1.03E+04			
VC-30'	1.13E+04		1.41E+04			

a. < and > symbols indicate a detection limit point

Table XVIII. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 3.0 mg/L at pH 9.5 and 25C in worst case water.a

AVG Cl2 CONCENTRATION 0'= 3.0 mg/L AVG HUMIC-FULVIC ACID CONC = 10.4 mg/L
30'= 1.3 mg/L AVG BENTONITE CLAY CONC = 5.05 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 1.19E-03	6.67E+00	9.74E-04	< 1.08E-03	> -2.97
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	5.60E+03		6.85E+03			
VC-0'	5.30E+03		6.70E+03			
VC-60'	5.90E+03		7.00E+03			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	7.80E+02	8.91E-02	6.70E+02	6.54E-02	7.73E-02	-1.11
TS-1'	< 6.67E+00	< 7.62E-04	< 6.67E+00	< 6.51E-04	< 7.07E-04	> -3.15
TS-3'						
TS-10'						
TS-30'						
AVG VC	8.75E+03		1.03E+04			
VC-0'	7.50E+03		1.30E+04			
VC-60'	1.00E+04		7.50E+03			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.30E+04	2.07E+00	2.44E+04	2.22E+00	2.15E+00	0.33
TS-1'	2.70E+04	2.43E+00	1.94E+04	1.76E+00	2.10E+00	0.32
TS-3'	3.53E+02	3.18E-02	4.00E+01	3.64E-03	1.77E-02	-1.75
TS-10'	< 6.67E+00	< 6.01E-04	< 6.67E+00	< 6.06E-04	< 6.04E-04	> -3.22
TS-30'						
AVG VC	1.11E+04		1.10E+04			
VC-0'	1.02E+04		1.09E+04			
VC-60'	1.20E+04		1.11E+04			

a. < and > symbols indicate a detection limit point.

Table XIX. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 7.0 mg/L at pH 4.5 and 5C in worst case water.a

AVG Cl2 CONCENTRATION 0'= 7.25 mg/L AVG HUMIC-FULVIC ACID CONC = 10.4 mg/L
30'= 6.42 mg/L AVG BENTONITE CLAY CONC = 5.0 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.93E+02	5.85E-03	8.70E+01	4.35E-03	5.10E-03	-2.29
TS-1'	< 6.67E+00	< 2.02E-04	< 6.67E+00	< 3.34E-04	< 2.68E-04	> -3.57
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC-WC	3.30E+04		2.00E+04			
VC-WC 0'	3.60E+04		2.20E+04	VC-HDF 0'	6.60E+03	4.70E+03
VC-WC 60'	3.00E+04		1.80E+04	VC-HDF 60'	2.87E+03	7.30E+03

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.27E+03	3.17E-02	2.60E+01	4.68E-04	1.61E-02	-1.79
TS-1'	< 6.67E+00	< 9.33E-05	< 6.67E+00	< 1.20E-04	< 1.07E-04	> -3.97
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC-WC	7.15E+04		5.55E+04			
VC-WC 0'	8.80E+04		6.40E+04	VC-HDF 0'	8.30E+04	1.87E+04
VC-WC 60'	5.50E+04		4.70E+04	VC-HDF 60'	2.13E+04	5.30E+03

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.33E+01	9.85E-04	< 6.67E+00	< 2.41E-04	6.13E-04	-3.21
TS-1'	< 6.67E+00	< 4.94E-04			< 2.47E-04	> -3.61
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC-WC	1.35E+04		2.77E+04			
VC-WC 0'	1.30E+04		3.13E+04	VC-HDF 0'	1.26E+04	9.30E+03
VC-WC 60'	1.40E+04		2.40E+04	VC-HDF 60'	4.50E+03	4.00E+03

a. < and > symbols indicate a detection limit point.

Table XX. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 7.0 mg/L at pH 4.5 and 25C in worst case water.a

AVG Cl2 CONCENTRATION 0'= 7.4 mg/L AVG HUMIC-FULVIC ACID CONC = 10.5 mg/L
30'= 5.5 mg/L AVG BENTONITE CLAY CONC = 5.1 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 2.72E-04	< 6.67E+00	< 4.89E-04	< 3.80E-04	> -3.42
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	2.45E+04		1.37E+04			
VC-0'	2.40E+04		1.80E+04			
VC-30'	2.50E+04		9.30E+03			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 4.60E-04	< 6.67E+00	< 4.39E-04	< 4.49E-04	> -3.35
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	1.45E+04		1.52E+04			
VC-0'	1.50E+04		1.90E+04			
VC-30'	1.40E+04		1.14E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 5.44E-04	< 6.67E+00	< 6.70E-04	< 6.07E-04	> -3.22
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	1.23E+04		9.95E+03			
VC-0'	1.15E+04		8.90E+03			
VC-30'	1.30E+04		1.10E+04			

a. < and > symbols indicate a detection limit point.

Table XXI. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 7.0 mg/L at pH 7.0 and 5C in worst case water.a

AVG Cl2 CONCENTRATION 0'= 7.2 mg/L 30'= 5.3 mg/L
AVG HUMIC-FULVIC ACID CONC = 10.3 mg/l
AVG BENTONITE CLAY CONC = 5.1 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.67E+01	6.88E-03	6.67E+01	8.04E-04	3.84E-03	-2.42
TS-1'	6.67E+00	9.83E-04	6.67E+00	8.04E-05	5.32E-04	-3.27
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC-WC	6.79E+03		8.30E+04			
VC-WC 0'	7.30E+03		8.50E+04	VC-HDF 0'	3.44E+04	3.00E+04
VC-WC 60'	6.27E+03		8.10E+04	VC-HDF 60'	2.00E+04	7.27E+04

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.70E+01	5.25E-04	1.33E+01	2.74E-04	4.00E-04	-3.40
TS-1'	< 6.67E+00	< 7.45E-05	< 6.67E+00	< 1.38E-04	< 1.06E-04	> -3.97
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC-WC	8.95E+04		4.85E+04			
VC-WC 0'	9.60E+04		5.60E+04	VC-HDF 0'	4.06E+04	4.80E+04
VC-WC 60'	8.30E+04		4.10E+04	VC-HDF 60'	6.67E+04	5.47E+04

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.00E+01	1.10E-03	2.00E+02	5.00E-03	3.05E-03	-2.52
TS-1'	< 6.67E+00	< 1.83E-04	< 6.67E+00	< 1.67E-04	< 1.75E-04	> -3.76
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC-WC	3.65E+04		4.00E+04			
VC-WC 0'	4.00E+04		5.00E+04	VC-HDF 0'	3.30E+04	4.80E+04
VC-WC 60'	3.30E+04		3.00E+04	VC-HDF 60'	4.00E+04	3.13E+04

a. < and > symbols indicate a detection limit point.

Table XXII. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 7.0 mg/L at pH 7.0 and 25C in worst case water.a

AVG Cl2 CONCENTRATION 0'= 6.69 mg/L AVG HUMIC-FULVIC ACID CONC = 10.4 mg/L
30'= 5.54 mg/L AVG BENTONITE CLAY CONC = 5.1 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 1.63E-04	< 6.67E+00	< 1.40E-04	< 1.52E-04	> -3.82
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	4.10E+04		4.75E+04			
VC-0'	3.80E+04		5.50E+04			
VC-30'	4.40E+04		4.00E+04			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 3.47E-04	< 6.67E+00	< 4.17E-04	< 3.82E-04	> -3.42
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	1.92E+04		1.60E+04			
VC-0'	2.03E+04		1.70E+04			
VC-30'	1.81E+04		1.50E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 7.06E-05	< 6.67E+00	< 5.80E-05	< 6.43E-05	> -4.19
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	9.45E+04		1.15E+05			
VC-0'	8.70E+04		1.20E+05			
VC-30'	1.02E+05		1.10E+05			

a. < and > symbols indicate a detection limit point.

Table XXIII. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 7.0 mg/L at pH 9.5 and 5C in worst case water.a

AVG Cl/2 CONCENTRATION 0'=5.3 mg/L AVG HUMIC-FULVIC ACID CONC= 10.4 mg/l
30'= 3.3 mg/L AVG BENTONITE CLAY CONC = 5.0 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	3.50E+02	9.21E-03	2.27E+01	4.22E-04	4.82E-03	-2.32
TS-1'	< 6.67E+00	< 1.76E-04	< 6.67E+00	< 1.24E-04	< 1.50E-04	> -3.82
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC-WC	3.80E+04		5.39E+04			
VC-WC 0'	3.60E+04		6.30E+04	VC-HDF 0'	2.80E+04	1.47E+04
VC-WC 60'	4.00E+04		4.47E+04	VC-HDF 60'	2.87E+04	1.93E+04

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.57E+04	1.97E-01	8.90E+03	8.64E-02	1.42E-01	-0.85
TS-1'	2.67E+02	3.35E-03	6.00E+01	5.83E-04	1.97E-03	-2.71
TS-3'	< 6.67E+00	< 8.37E-05	< 6.67E+00	< 6.48E-05	< 7.42E-05	> -4.13
TS-10'						
TS-30'						
TS-60'						
AVG VC-WC	7.97E+04		1.03E+05			
VC-WC 0'	9.07E+04		1.05E+05	VC-HDF 0'	7.87E+04	9.80E+04
VC-WC 60'	6.87E+04		1.01E+05	VC-HDF 60'	8.44E+04	8.27E+04

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	5.27E+05	1.02E+01	1.72E+05	3.18E+00	6.69E+00	0.83
TS-1'	1.07E+05	2.07E+00	2.73E+04	5.04E-01	1.29E+00	0.11
TS-3'	8.67E+01	1.68E-03	5.30E+01	9.79E-04	1.33E-03	-2.88
TS-10'	< 6.67E+00	< 1.29E-04	< 6.67E+00	< 1.23E-04	< 1.26E-04	> -3.90
TS-30'						
TS-60'						
AVG VC-WC	5.17E+04		5.42E+04			
VC-WC 0'	5.20E+04		5.33E+04	VC-HDF 0'	4.67E+04	7.27E+04
VC-WC 60'	5.13E+04		5.50E+04	VC-HDF 60'	4.47E+04	5.67E+04

a. < and > symbols indicate a detection limit point.

Table XXIV. Inactivation of HAV, poliovirus 1, and echovirus 1 by an initial free chlorine dose of approximately 7.0 mg/L at pH 9.5 and 25C in worst case water.a

AVG Cl2 CONCENTRATION 0'= 6.32 mg/L AVG HUMIC-FULVIC ACID CONC = 10.4 mg/L
30'= 5.35 mg/L AVG BENTONITE CLAY CONC = 5.1 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 9.39E-04	< 6.67E+00	< 8.34E-04	< 8.87E-04	> -3.05
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	7.10E+03		8.00E+03			
VC-0'	7.50E+03		6.40E+03			
VC-30'	6.70E+03		9.60E+03			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 4.17E-04	< 6.67E+00	< 3.66E-04	< 3.92E-04	> -3.41
TS-1'						
TS-3'						
TS-10'						
TS-30'						
AVG VC	1.60E+04		1.82E+04			
VC-0'	1.90E+04		2.04E+04			
VC-30'	1.30E+04		1.60E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 6.67E+00	< 1.05E-04	2.00E+01	2.88E-04	1.96E-04	-3.71
TS-1'			< 6.67E+00	< 9.60E-05	< 4.80E-05	> -4.32
TS-3'						
TS-10'						
TS-30'						
AVG VC	6.35E+04		6.95E+04			
VC-0'	6.30E+04		5.70E+04			
VC-30'	6.40E+04		8.20E+04			

a. < and > symbols indicate a detection limit point.

Table XXV. Inactivation of HAV strain MD-1 by an initial free chlorine dose of approximately 1.0 mg/L at pH 4.5 and 5C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 1.08 mg/L
60'= 0.91 mg/L

VIRUS = HAV MD-1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.00E+04	1.05E-01	1.90E+04	1.46E-01	1.26E-01	-0.90
TS-1'	7.30E+02	3.84E-03	4.80E+02	3.69E-03	3.77E-03	-2.42
TS-3'	2.00E+01	1.05E-04	< 6.67E+00	< 5.13E-05	7.8?E-05	> -4.11
TS-10'	< 6.67E+00	< 3.51E-05			< 1.76E-05	> -4.76
TS-30'						
TS-60'						
AVG VC	1.90E+05		1.30E+05			
VC-0'	2.00E+05		1.00E+05			
VC-60'	1.75E+05		1.60E+05			

a. < and > symbols indicate a detection limit point.

Table XXVI. Inactivation of HAV strain MD-1 and echovirus 1 by an initial free chlorine dose of approximately 1.0 mg/L at pH 9.5 and 5C in halogen demand free water.a

AVG Cl2 CONCENTRATION 0'= 1.06 mg/L
60'= 0.9 mg/L

VIRUS = HAV MD-1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.20E+04	2.18E-01	8.90E+02	1.65E-02	1.17E-01	-0.93
TS-1'	2.00E+02	3.64E-03	< 6.67E+00	< 1.24E-04	< 1.88E-03	> -2.73
TS-3'	< 6.67E+00	< 1.21E-04			< 6.06E-05	> -4.22
TS-10'						
TS-30'						
TS-60'						
AVG VC	5.50E+04		5.39E+04			
VC-0'	1.60E+04		6.30E+04			
VC-60'	9.30E+04		4.47E+04			

VIRUS= ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	5.13E+04	1.30E+00	3.80E+04	9.92E-01	1.15E+00	0.06
TS-1'	9.33E+04	2.36E+00	9.07E+04	2.37E+00	2.37E+00	0.37
TS-3'	< 3.50E+05	8.86E+00	3.60E+05	9.40E+00	9.13E+00	0.96
TS-10'	4.07E+05	1.03E+01	4.80E+05	1.25E+01	1.14E+01	1.06
TS-30'	1.60E+05	4.05E+00	1.39E+05	3.63E+00	3.84E+00	0.58
TS-60'	5.73E+04	1.45E+00	1.28E+04	3.34E-01	8.92E-01	-0.05
AVG VC	3.95E+04		3.83E+04			
VC-0'	2.[illegible]E+04		3.53E+04			
VC-60'	5[illegible]14		4.13E+04			

a. < and > symbols indicate a detection limit point.

Table A27

Inactivation of HAV Strain CR326 by 1 mg/L Free Chlorine at pH 4.5 and 5°C.

Avg Cl2 Concentration			T0 = 1.86 mg/L T60= 0.96 mg/L			
Virus = HAV CR326						
Sample	PFU/mL	Nt/No	PFU/mL	Nt/No	Avg Nt/No	Avg Log Nt/No
TS-20"	2.20E4	2.75E-2	2.21E4	1.26E-1	2.01E-2	-1.11
TS-1'	1.23E3	1.53E-3	6.23E2	3.56E-3	2.55E-3	-2.59
TS-3'	2.11E2	2.64E-4	7.77E1	4.44E-4	3.54E-4	-3.45
TS-10'	1.11E1	1.39E-5	1.11E1	6.34E-5	3.87E-5	-4.41
TS-30'	0.00	---	0.00	---	---	-----
TS-60'	0.00	---	0.00	---	---	-----
Avg VC	6.84E5		1.92E5			
VC-0'	8.00E5		1.75E5			
VC-60'	5.67E5		2.08E5			

Inactivation of HAV Strain CR326 by 1 mg/L Free Chlorine at pH 9.5 and 5°C

Avg Cl2 Concentration			T0 = 1.72 mg/L T60 = 1.05 mg/L			
Virus = HAV CR326						
Sample	PFU/mL	Nt/No	PFU/mL	Nt/No	Avg Nt/No	Avg Log Nt/No
TS-20"	3.27E5	2.80E-1	2.08E5	5.32E-1	4.06E-1	-0.39
TS-1'	1.29E4	1.10E-2	2.73E4	6.99E-2	4.04E-2	-1.39
TS-3'	2.17E3	1.86E-3	8.00E2	2.05E-3	1.95E-3	-2.71
TS-10'	2.33E2	1.99E-4	2.76E2	7.06E-4	4.53E-4	-3.34
TS-30'	1.11E1	9.49E-6	1.11E1	2.84E-5	1.89E-5	-4.72
TS-60'	0.00	---	0.00	---	---	-----
Avg VC	1.24E6		3.87E5			
VC-0'	1.17E6		3.91E5			
VC-60'	1.30E6		3.83E5			

Table A28

IMACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 4.5 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 8.72 MG/L
60' = 7.13 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	3.47E+03	3.47E-01	1.80E+04	1.68E-01	2.58E-01	-0.59
TS-1'	1.60E+03	1.60E-01	1.73E+04	1.62E-01	1.61E-01	-0.79
TS-3'	1.20E+03	1.20E-01	1.47E+04	1.37E-01	1.29E-01	-0.89
TS-10'	6.80E+02	6.80E-02	3.07E+03	2.87E-02	4.83E-02	-1.32
TS-30'	< 6.67E+00	< 6.67E-04	< 6.67E+00	< 6.23E-05	< 3.65E-04	> -3.44
TS-60'						
VC-0'	1.00E+04		1.07E+05			
VC-60'	9.33E+03		1.00E+05			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	5.13E+03	7.69E-01	8.80E+03	6.29E-01	6.99E-01	-0.16
TS-1'	5.73E+03	8.59E-01	8.33E+03	5.95E-01	7.27E-01	-0.14
TS-3'	7.26E+03	1.09E+00	7.80E+03	5.57E-01	8.23E-01	-0.08
TS-10'	3.73E+03	5.59E-01	9.33E+03	6.66E-01	6.13E-01	-0.21
TS-30'	3.13E+03	4.69E-01	8.80E+03	6.29E-01	5.49E-01	-0.26
TS-60'	1.67E+03	2.50E-01	5.73E+03	4.09E-01	3.30E-01	-0.48
VC-0'	6.67E+03		1.40E+04			
VC-60'	6.13E+03		1.20E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.00E+03	1.54E-01	2.80E+03	2.48E-01	2.01E-01	-0.70
TS-1'	2.00E+03	1.54E-01	4.80E+03	4.25E-01	2.89E-01	-0.54
TS-3'	6.00E+03	4.62E-01	1.03E+04	9.12E-01	6.87E-01	-0.16
TS-10'	2.27E+04	1.75E+00	1.53E+04	1.35E+00	1.55E+00	0.19
TS-30'	2.60E+04	2.00E+00	1.30E+04	1.15E+00	1.58E+00	0.20
TS-60'	2.20E+02	1.69E-02	2.00E+02	1.77E-02	1.73E-02	-1.76
VC-0'	1.30E+04		1.13E+04			
VC-60'	2.33E+04		9.60E+03			

Table A29

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 7.0 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 8.58 MG/L
60' = 7.29 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.07E+03	1.65E-02	9.87E+03	1.23E-01	7.00E-02	-1.16
TS-1'	< 6.67E+00	< 1.03E-04	1.33E+02	1.66E-03	< 8.83E-04	> -3.05
TS-3'	< 6.67E+00	< 1.03E-04	< 6.67E+00	< 8.34E-05	< 9.32E-05	> -4.03
TS-10'						
TS-30'						
TS-60'						
VC-0'	6.47E+04		8.00E+04			
VC-60'	6.27E+04		4.40E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.31E+04	3.71E-01	4.60E+03	5.75E-01	4.73E-01	-0.33
TS-1'	1.19E+04	3.37E-01	2.13E+03	2.66E-01	3.02E-01	-0.52
TS-3'	7.07E+03	2.00E-01	2.20E+03	2.75E-01	2.38E-01	-0.62
TS-10'	5.93E+03	1.68E-01	1.25E+03	1.56E-01	1.62E-01	-0.79
TS-30'	3.07E+03	8.70E-02	5.00E+02	6.25E-02	7.47E-02	-1.13
TS-60'	5.20E+02	1.47E-02	4.00E+01	5.00E-03	9.87E-03	-2.01
VC-0'	3.53E+04		8.00E+03			
VC-60'	2.29E+04		7.87E+03			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.03E+03	8.73E-02	8.00E+03	4.57E-01	2.72E-01	-0.57
TS-1'	4.53E+03	3.84E-01	1.14E+04	6.51E-01	5.18E-01	-0.29
TS-3'	1.18E+04	1.00E+00	5.60E+04	3.20E+00	2.10E+00	0.32
TS-10'	1.07E+02	9.07E-03	5.60E+04	3.20E+00	1.60E+00	0.21
TS-30'	< 6.67E+00	< 5.65E-04	< 6.67E+00	< 3.81E-04	< 4.73E-04	> -3.32
TS-60'						
VC-0'	1.18E+04		1.75E+04			
VC-60'	9.20E+03		1.49E+04			

Table A30

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 9.5 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 8.84 MG/L
30' = 6.30 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 4.54E-04	< 6.67E+00	< 8.13E-05	< 2.68E-04	> -3.57
TS-1'						
TS-3'						
TS-10'						
TS-30'						
VC-0'	1.47E+04		8.40E+04			
VC-30	2.73E+04		8.20E+04 = VC-60			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	3.87E+03	8.80E-02	9.93E+02	2.61E-02	5.70E-02	-1.24
TS-1'	1.20E+02	2.73E-03	8.00E+01	2.11E-03	2.42E-03	-2.62
TS-3'	3.33E+01	7.57E-04	< 6.67E+00	< 1.76E-04	< 4.66E-04	> -3.33
TS-10'	< 6.67E+00	< 1.52E-04	< 6.67E+00	< 1.76E-04	< 1.64E-04	> -3.79
TS-30'						
VC-0'	4.40E+04		3.80E+04			
VC-30	7.20E+04		2.00E+04 = VC-60			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.67E+05	9.65E+00	1.09E+04	4.80E+00	7.23E+00	0.86
TS-1'	2.20E+03	1.27E-01	1.46E+03	6.43E-01	3.85E-01	-0.41
TS-3'	< 6.67E+00	< 3.86E-04	< 6.67E+00	< 2.94E-03	< 1.66E-03	> -2.78
TS-10'						
TS-30'						
VC-0'	1.73E+04		2.27E+03			
VC-30	3.67E+04		1.98E+03 = VC-60			

Table A31

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 4.5 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 8.03 MG/L
60' = 6.24 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	6.13E+02	6.61E-02	1.33E+03	1.32E-01	9.89E-02	-1.00
TS-1'	1.80E+03	1.94E-01	1.33E+03	1.32E-01	1.63E-01	-0.79
TS-3'	1.60E+02	1.73E-02	1.10E+02	1.09E-02	1.41E-02	-1.85
TS-10'	< 6.67E+00	< 7.20E-04	< 6.67E+00	< 6.60E-04	< 6.90E-04	> -3.16
TS-30'						
TS-60'						
VC-0'	9.27E+03		1.01E+04			
VC-60'	6.33E+03		1.02E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.00E+03	2.38E-01	1.45E+04	3.69E-01	3.04E-01	-0.52
TS-1'	3.33E+03	3.96E-01	1.45E+04	3.69E-01	3.83E-01	-0.42
TS-3'	1.47E+03	1.75E-01	1.45E+04	3.69E-01	2.72E-01	-0.57
TS-10'	9.33E+02	1.11E-01	4.47E+03	1.14E-01	1.12E-01	-0.95
TS-30'	< 6.67E+00	< 7.94E-04	< 6.67E+00	< 1.70E-04	< 4.82E-04	> -3.32
TS-60'						
VC-0'	8.40E+03		3.93E+04			
VC-60'	7.00E+03		5.60E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	7.20E+03	8.37E-01	8.87E+03	2.29E-01	5.33E-01	-0.27
TS-1'	7.07E+03	8.22E-01	5.00E+04	1.29E+00	1.06E+00	0.02
TS-3'	6.00E+03	6.98E-01	5.47E+04	1.41E+00	1.06E+00	0.02
TS-10'	6.67E+00	7.76E-04	< 6.67E+00	< 1.72E-04	< 4.74E-04	> -3.32
TS-30'	< 6.67E+00	< 7.76E-04	< 6.67E+00	< 1.72E-04	< 4.74E-04	> -3.32
TS-60'						
VC-0'	8.60E+03		3.87E+04			
VC-60'	8.53E+03		1.01E+04			

Table A32

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 7.0 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 8.51 MG/L
60' = 7.71 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 7.76E-04	< 6.67E+00	< 7.67E-05	< 4.26E-04	> -3.37
TS-1'						
TS-3'						
TS-10'						
TS-30'						
TS-60'						
VC-0'	8.60E+03		8.70E+04			
VC-60'	6.80E+03		9.60E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	7.13E+03	7.75E-01	1.74E+04	1.18E+00	9.75E-01	-0.01
TS-1'	3.00E+03	3.26E-01	1.90E+04	1.28E+00	8.05E-01	-0.09
TS-3'	6.67E+00	7.25E-04	< 6.67E+00	< 4.51E-04	< 5.88E-04	> -3.23
TS-10'	< 6.67E+00	< 7.25E-04	< 6.67E+00	< 4.51E-04	< 5.88E-04	> -3.23
TS-30'						
TS-60'						
VC-0'	9.20E+03		1.48E+04			
VC-60'	8.93E+03		2.10E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	6.33E+03	6.93E-01	2.15E+03	1.95E+00	1.32E+00	0.12
TS-1'	< 6.67E+00	< 7.31E-04	1.80E+03	1.64E+00	< 8.19E-01	> -0.09
TS-3'	< 6.67E+00	< 7.31E-04	< 6.67E+00	< 6.06E-03	< 3.40E-03	> -2.47
TS-10'						
TS-30'						
TS-60'						
VC-0'	9.13E+03		1.10E+03			
VC-60'	8.73E+03		1.48E+03			

Table A33

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 9.5 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 8.00 MG/L
60' = 1.57 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 2.67E-05	< 6.67E+00	< 5.85E-05	< 4.26E-05	> -4.37
TS-1'						
TS-3'						
TS-10'						
TS-30'						
TS-60'						
VC-0'	2.50E+05		1.14E+05			
VC-60'	2.47E+05		1.21E+05			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	3.53E+02	1.68E-02	9.00E+01	3.33E-03	1.01E-02	-2.00
TS-1'	5.33E+01	2.54E-03	< 6.67E+00	< 2.47E-04	< 1.39E-03	> -2.86
TS-3'	2.00E+01	9.52E-04	< 6.67E+00	< 2.47E-04	< 6.00E-04	> -3.22
TS-10'	< 6.67E+00	< 3.18E-04	< 6.67E+00	< 2.47E-04	< 2.82E-04	> -3.55
TS-30'						
TS-60'						
VC-0'	2.10E+04		2.70E+04			
VC-60'	1.14E+04		3.10E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	5.47E+03	6.84E-01	3.30E+03	3.03E+00	1.86E+00	0.27
TS-1'	< 6.67E+00	< 8.34E-04	< 6.67E+00	< 6.12E-03	< 3.48E-03	> -2.46
TS-3'						
TS-10'						
TS-30'						
TS-60'						
VC-0'	8.00E+03		1.09E+03			
VC-60'	1.27E+04		5.10E+03			

Table A34

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 4.5 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 17.63 MG/L
30' = 16.70 MG/L
60' = 16.46 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.73E+03	1.86E-01	4.80E+03	3.36E-01	2.61E-01	-0.58
TS-1'	3.00E+03	2.04E-01	3.53E+03	2.47E-01	2.25E-01	-0.65
TS-3'	1.25E+03	8.50E-02	1.53E+03	1.07E-01	9.60E-02	-1.02
TS-10'	1.13E+03	7.69E-02	5.87E+02	4.10E-02	5.90E-02	-1.23
TS-30'	1.70E+01	1.16E-03	1.53E+02	1.07E-02	5.93E-03	-2.23
TS-60'	1.33E+00	9.05E-05	2.00E+00	1.40E-04	1.15E-04	-3.94
VC-0'	1.47E+04		1.43E+04			
VC-60'	1.29E+04		1.50E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.93E+04	2.14E-01	2.47E+04	2.35E-01	2.25E-01	-0.65
TS-1'	2.27E+04	2.52E-01	3.40E+04	3.24E-01	2.88E-01	-0.54
TS-3'	1.63E+04	1.81E-01	2.93E+04	2.79E-01	2.30E-01	-0.64
TS-10'	1.28E+04	1.42E-01	2.13E+04	2.03E-01	1.73E-01	-0.76
TS-30'	7.80E+03	8.67E-02	1.08E+04	1.03E-01	9.48E-02	-1.02
TS-60'	1.40E+03	1.56E-02	1930.00	1.84E-02	1.70E-02	-1.77
VC-0'	9.00E+04		1.05E+05			
VC-60'	9.07E+04		9.93E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	5.60E+03	5.00E-02	9.33E+03	9.15E-02	7.07E-02	-1.15
TS-1'	2.00E+04	1.79E-01	3.53E+04	3.46E-01	2.62E-01	-0.58
TS-3'	7.07E+04	6.31E-01	9.33E+04	9.15E-01	7.73E-01	-0.11
TS-10'	2.20E+05	1.96E+00	1.00E+05	9.80E-01	1.47E+00	0.17
TS-30'	3.53E+03	3.15E-02	2.47E+03	2.42E-02	2.79E-02	-1.55
TS-60'	6.67E+00	5.95E-05	6.67E+00	6.54E-05	6.25E-05	-4.20
VC-0'	1.12E+05		1.02E+05			
VC-60'	4.27E+04		1.17E+05			

Table A35

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 7.0 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 17.74 MG/L
60' = 16.25 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	8.20E+02	1.58E-02	1.00E+05	2.78E+00	1.40E+00	0.15
TS-1'	< 6.67E+00	< 1.28E-04	< 6.67E+00	< 1.85E-04	< 1.57E-04	> -3.80
TS-3'						
TS-10'						
TS-30'						
TS-60'						
VC-0'	5.20E+04		3.60E+04			
VC-60'	7.00E+04		4.13E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.67E+03	4.17E-01	1.36E+04	2.22E-01	3.20E-01	-0.50
TS-1'	2.20E+03	3.44E-01	1.35E+04	2.20E-01	2.82E-01	-0.55
TS-3'	2.20E+03	3.44E-01	1.31E+04	2.14E-01	2.79E-01	-0.55
TS-10'	1.47E+03	2.30E-01	1.39E+04	2.27E-01	2.28E-01	-0.64
TS-30'	1.27E+02	1.98E-02	2.73E+03	4.45E-02	3.22E-02	-1.49
TS-60'	< 6.67E+00	< 1.04E-03	< 6.67E+00	< 1.09E-04	< 5.75E-04	> -3.24
VC-0'	6.40E+03		6.13E+04			
VC-60'	7.80E+03		7.67E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.33E+04	5.72E-01	6.40E+03	9.85E-02	3.35E-01	-0.47
TS-1'	5.00E+04	1.23E+00	4.20E+04	6.46E-01	9.37E-01	-0.03
TS-3'	7.53E+03	1.85E-01	3.93E+04	6.05E-01	3.95E-01	-0.40
TS-10'	< 6.67E+00	< 1.64E-04	4.00E+01	6.15E-04	< 3.90E-04	> -3.41
TS-30'	< 6.67E+00	< 1.64E-04	< 6.67E+00	< 1.03E-04	< 1.33E-04	> -3.88
TS-60'						
VC-0'	4.07E+04		6.50E+04			
VC-60'	6.80E+04		7.20E+04			

Table A36

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 9.5 AND 5 C

AVERAGE I2 CONCENTRATION: 0' = 16.01 MG/L
30' = 13.43 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 1.32E-04	< 6.67E+00	< 1.64E-04	< 1.48E-04	> -3.83
TS-1'						
TS-3'						
TS-10'						
TS-30'						
VC-0'	5.07E+04		4.07E+04			
VC-30	5.40E+04		4.47E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	8.33E+03	7.24E-02	6.93E+03	5.54E-02	6.39E-02	-1.19
TS-1'	4.67E+02	4.06E-03	4.07E+02	3.26E-03	3.66E-03	-2.44
TS-3'	6.67E+01	7.54E-04	6.67E+01	5.34E-04	6.44E-04	-3.19
TS-10'	2.67E+01	2.32E-04	2.67E+01	2.14E-04	2.23E-04	-3.65
TS-30'	< 6.67E+00	< 5.80E-05	< 6.67E+00	< 5.34E-05	< 5.57E-05	> -4.25
VC-0'	1.15E+05		1.25E+05			
VC-30	1.16E+05		1.15E+05			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.80E+05	1.43E+00	1.73E+05	1.92E+00	1.68E+00	0.22
TS-1'	3.07E+03	2.44E-02	5.20E+02	5.78E-03	1.51E-02	-1.82
TS-3'	4.00E+01	3.17E-04	< 6.67E+00	< 7.41E-05	< 1.96E-04	> -3.71
TS-10'	1.33E+01	1.06E-04	< 6.67E+00	< 7.41E-05	< 8.98E-05	> -4.05
TS-30'	< 6.67E+00	< 5.29E-05	< 6.67E+00	< 7.41E-05	< 6.35E-05	> -4.20
VC-0'	1.26E+05		9.00E+04			
VC-30	1.30E+05		9.40E+04			

Table A37

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 4.5 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 17.18 MG/L
30' = 15.16 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	4.13E+03	2.70E-01	3.60E+03	2.25E-01	2.47E-01	-0.61
TS-1'	1.47E+03	9.61E-02	2.60E+03	1.63E-01	1.29E-01	-0.89
TS-3'	2.67E+02	1.75E-02	4.67E+02	2.92E-02	2.33E-02	-1.63
TS-10'	< 6.67E+00	< 4.36E-04	< 6.67E+00	< 4.17E-04	< 4.26E-04	> -3.37
TS-30'						
VC-0'	1.53E+04		1.69E+04			
VC-30	2.07E+04		2.06E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	5.73E+03	4.58E-01	6.67E+03	6.29E-01	5.44E-01	-0.26
TS-1'	5.07E+03	4.06E-01	8.80E+03	8.30E-01	6.18E-01	-0.21
TS-3'	2.80E+03	2.24E-01	4.73E+03	4.46E-01	3.35E-01	-0.47
TS-10'	< 6.67E+00	< 5.34E-04	6.67E+00	6.29E-04	< 5.81E-04	> -3.24
TS-30'	< 6.67E+00	< 5.34E-04	< 6.67E+00	< 6.29E-04	< 5.81E-04	> -3.24
VC-0'	1.25E+04		1.06E+04			
VC-30	1.23E+04		1.45E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.19E+04	1.19E+00	1.05E+04	7.50E-01	9.70E-01	-0.01
TS-1'	4.13E+04	4.13E+00	4.80E+04	3.43E+00	3.78E+00	0.58
TS-3'	1.30E+02	1.30E-02	3.33E+02	2.38E-02	1.84E-02	-1.74
TS-10'	< 6.67E+00	< 6.67E-04	1.33E+01	9.50E-04	< 8.09E-04	> -3.09
TS-30'	< 6.67E+00	< 6.67E-04	< 6.67E+00	< 4.76E-04	< 5.72E-04	> -3.24
VC-0'	1.00E+04		1.40E+04			
VC-30	1.10E+04		1.40E+04			

Table A38

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 7.0 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 16.51 MG/L
30' = 13.21 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 2.38E-04	< 6.67E+00	< 2.38E-04	< 4.77E-04	> -3.32
TS-1'						
TS-3'						
TS-10'						
TS-30'						
VC-0'	3.13E+04		9.00E+03			
VC-30	3.07E+04		1.40E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	4.40E+03	1.57E-01	8.80E+02	1.65E-01	1.61E-01	-0.79
TS-1'	2.53E+03	9.04E-02	3.07E+02	5.76E-02	7.40E-02	-1.13
TS-3'	2.00E+01	7.14E-04	6.67E+00	1.25E-03	9.83E-04	-3.01
TS-10'	< 6.67E+00	< 2.38E-04	< 6.67E+00	< 1.25E-03	< 7.45E-04	> -3.13
TS-30'						
VC-0'	2.80E+04		5.33E+03			
VC-30	2.33E+04		5.86E+03			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	1.01E+05	4.21E+00	1.70E+04	7.52E-01	2.48E+00	0.39
TS-1'	1.30E+01	5.42E-04	< 6.67E+00	< 2.95E-04	< 4.18E-04	> -3.38
TS-3'	< 6.67E+00	< 2.78E-04	< 6.67E+00	< 2.95E-04	< 2.87E-04	> -3.54
TS-10'						
TS-30'						
VC-0'	2.40E+04		2.26E+04			
VC-30	2.80E+04		2.80E+04			

Table A39

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 9.5 AND 25 C

AVERAGE I2 CONCENTRATION: 0' = 16.52 MG/L
30' = 2.20 MG/L

VIRUS = HEPATITIS A

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 2.64E-04	< 6.67E+00	< 2.22E-04	< 2.43E-04	> -3.61
TS-1'						
TS-3'						
TS-10'						
TS-30'						
VC-0'	2.53E+04		3.00E+04			
VC-30	2.26E+04		4.67E+04			

VIRUS = POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	< 6.67E+00	< 6.72E-04	6.67E+00	1.57E-03	< 1.12E-03	> -2.95
TS-1'	< 6.67E+00	< 6.72E-04	< 6.67E+00	< 1.57E-03	< 1.12E-03	> -2.95
TS-3'						
TS-10'						
TS-30'						
VC-0'	9.93E+03		4.26E+03			
VC-30	9.93E+03		3.33E+03			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU/ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20''	2.40E+02	3.43E-03	3.47E+02	5.10E-03	4.27E-03	-2.37
TS-1'	< 6.67E+00	< 9.53E-05	< 6.67E+00	< 9.81E-05	< 9.67E-05	> -4.01
TS-3'						
TS-10'						
TS-30'						
VC-0'	7.00E+04		6.80E+04			
VC-30	7.00E+04		6.40E+04			

Table A40

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 4.5 AND 5C
IN WORST CASE WATER

AVG I2 CONCENTRATION 0'= 8.8 mg/L AVG HUMIC-FULVIC CONC= 9.9 mg/L
60'= 5.2 mg/L AVG BENTONITE CLAY= 5.4 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.09E+03	1.53E+00	1.13E+03	9.46E-01	1.24E+00	0.09
TS-1'	5.07E+02	7.11E-01	4.53E+02	3.79E-01	5.45E-01	-0.26
TS-3'	2.93E+02	4.11E-01	3.87E+02	3.24E-01	3.67E-01	-0.43
TS-10'	1.27E+02	1.78E-01	1.13E+02	9.46E-02	1.36E-01	-0.87
TS-30'	8.67E+01	1.22E-01	8.00E+01	6.69E-02	9.43E-02	-1.03
TS-60'	1.33E+01	1.87E-02	2.00E+01	1.67E-02	1.77E-02	-1.75
AVG VC	7.13E+02		1.20E+03			
VC-0'	6.66E+02		1.25E+03			
VC-60'	7.60E+02		1.14E+03			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	3.53E+03	5.46E-01	4.07E+03	5.05E-01	5.25E-01	-0.28
TS-1'	4.13E+03	6.38E-01	3.93E+03	4.87E-01	5.63E-01	-0.25
TS-3'	3.67E+03	5.67E-01	4.40E+03	5.46E-01	5.56E-01	-0.25
TS-10'	3.53E+03	5.46E-01	3.60E+03	4.46E-01	4.96E-01	-0.30
TS-30'	4.40E+03	6.80E-01	3.60E+03	4.46E-01	5.63E-01	-0.25
TS-60'	2.80E+03	4.33E-01	2.40E+03	2.98E-01	3.65E-01	-0.44
AVG VC	6.47E+03		8.07E+03			
VC-0'	6.47E+03		7.80E+03			
VC-60'	6.47E+03		8.33E+03			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.21E+03	1.11E-01	3.33E+03	4.60E-02	7.85E-02	-1.11
TS-1'	3.47E+03	3.18E-01	1.23E+03	1.70E-02	1.68E-01	-0.78
TS-3'	8.10E+03	7.43E-01	4.60E+03	6.36E-02	4.03E-01	-0.39
TS-10'	1.42E+04	1.30E+00	2.73E+04	3.77E-01	8.40E-01	-0.08
TS-30'	6.73E+03	6.17E-01	2.60E+04	3.59E-01	4.88E-01	-0.31
TS-60'	4.07E+03	3.73E-01	5.07E+03	7.01E-02	2.22E-01	-0.65
AVG VC	1.09E+04		7.24E+04			
VC-0'	1.18E+04		5.07E+04			
VC-60'	1.00E+04		9.40E+04			

Table A41

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 7.0 AND 5C
IN WORST CASE WATER

AVG I2 CONCENTRATION 0'= 8.8 mg/L AVG HUMIC-FULVIC CONC= 9.5 mg/L
60'= 4.9 mg/L AVG BENTONITE CLAY= 5.4 NTU

VIRUS = HAV

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.67E+03	2.75E-01	5.15E+02	1.16E-01	1.96E-01	-0.71
TS-1'	6.00E+01	9.89E-03	1.00E+01	2.25E-03	6.07E-03	-2.22
TS-3'	< 5.00E+00	< 8.24E-04	5.00E+00	1.13E-03	< 9.76E-04	> -3.01
TS-10'	5.00E+00	8.24E-04	5.00E+00	1.13E-03	9.76E-04	-3.01
TS-30'	2.00E+01	3.30E-03	5.00E+00	1.13E-03	2.21E-03	-2.66
TS-60'	5.00E+00	8.24E-04	< 5.00E+00	< 1.13E-03	< 9.76E-04	> -3.01
AVG VC	6.07E+03		4.44E+03			
VC-0'	6.00E+03		3.07E+03			
VC-60'	6.13E+03		5.80E+03			

VIRUS= POLIO 1

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.07E+04	3.57E-01	1.71E+04	4.07E-01	3.82E-01	-0.42
TS-1'	8.67E+03	2.89E-01	1.43E+04	3.40E-01	3.15E-01	-0.50
TS-3'	1.15E+04	3.83E-01	1.29E+04	3.07E-01	3.45E-01	-0.46
TS-10'	8.00E+03	2.67E-01	1.14E+04	2.71E-01	2.69E-01	-0.57
TS-30'	7.73E+03	2.58E-01	9.13E+03	2.17E-01	2.38E-01	-0.62
TS-60'	3.87E+03	1.29E-01	4.27E+03	1.02E-01	1.15E-01	-0.94
AVG VC	3.00E+04		4.20E+04			
VC-0'	3.13E+04		4.67E+04			
VC-60'	2.87E+04		3.73E+04			

VIRUS = ECHO 1

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	5.05E+02	6.45E-03	7.85E+02	8.21E-03	7.33E-03	-2.14
TS-1'	1.93E+03	2.46E-02	5.93E+03	6.20E-02	4.33E-02	-1.36
TS-3'	2.60E+04	3.32E-01	4.87E+04	5.09E-01	4.20E-01	-0.38
TS-10'	3.27E+04	4.17E-01	4.33E+04	4.53E-01	4.35E-01	-0.36
TS-30'	9.00E+01	1.15E-03	4.00E+01	4.18E-04	7.83E-04	-3.11
TS-60'	< 5.00E+00	< 6.38E-05	1.00E+01	1.05E-04	> 8.42E-05	> -4.07
AVG VC	7.84E+04		9.57E+04			
VC-0'	8.07E+04		8.13E+04			
VC-60'	7.60E+04		1.10E+05			

Table A42

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 1 TABLET/QUART IODINE AT pH 9.5 AND 5C IN WORST CASE WATER

AVG I2 CONCENTRATION 0'= 9.3 mg/L AVG HUMIC-FULVIC CONC= 9.8 mg/L
60'= < 0.5 mg/L AVG BENTONITE CLAY= 4.9 NTU

VIRUS = HAV

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 2.00E+00	< 3.15E-04	< 2.00E+00	< 3.94E-04	< 3.55E-04	> -3.45
TS-1'	2.00E+00	3.15E-04	2.00E+00	3.94E-04	3.55E-04	-3.45
TS-3'	< 2.00E+00	< 3.15E-04	< 2.00E+00	< 3.94E-04	< 3.55E-04	> -3.45
TS-10'						
TS-30'						
TS-60'						
AVG VC	6.35E+03		5.07E+03			
VC-0'	7.00E+03		5.67E+03			
VC-60'	5.70E+03		4.47E+03			

VIRUS= POLIO 1

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	9.07E+02	7.31E-02	8.80E+02	6.54E-02	6.93E-02	-1.16
TS-1'	1.60E+02	1.29E-02	2.27E+02	1.69E-02	1.49E-02	-1.83
TS-3'	< 6.67E+00	< 5.38E-04	4.00E+01	2.97E-03	< 1.76E-03	> -2.76
TS-10'	< 6.67E+00	< 5.38E-04	1.33E+01	9.91E-04	< 7.64E-04	> -3.12
TS-30'	< 6.67E+00	< 5.38E-04	< 1.33E+01	< 9.91E-04	< 7.64E-04	> -3.12
TS-60'						
AVG VC	1.24E+04		1.35E+04			
VC-0'	1.32E+04		1.44E+04			
VC-60'	1.16E+04		1.25E+04			

VIRUS = ECHO 1

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	9.33E+04	1.23E+00	1.01E+05	1.08E+00	1.15E+00	0.06
TS-1'	3.20E+04	4.21E-01	1.04E+04	1.11E-01	2.66E-01	-0.57
TS-3'	4.00E+01	5.26E-04	4.00E+01	4.28E-04	4.77E-04	-3.32
TS-10'	< 1.33E+01	< 1.75E-04	1.33E+01	1.43E-04	< 1.59E-04	> -3.80
TS-30'	1.33E+01	1.75E-04	< 1.33E+01	< 1.43E-04	< 1.59E-04	> -3.80
TS-60'						
AVG VC	7.60E+04		9.35E+04			
VC-0'	6.67E+04		7.20E+04			
VC-60'	8.53E+04		1.15E+05			

Table A43

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 4.5 AND 5C
IN WORST CASE WATER

AVG I2 CONCENTRATION 0'= 16.4 mg/L AVG HUMIC-FULVIC CONC= 10.4 mg/L
60'= 13.4 mg/L AVG BENTONITE CLAY= 5.5 NTU

VIRUS = HAV

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.73E+03	6.77E-01	3.30E+03	9.34E-01	8.05E-01	-0.09
TS-1'	1.73E+03	4.29E-01	2.00E+03	5.66E-01	4.98E-01	-0.30
TS-3'	7.00E+02	1.74E-01	1.13E+03	3.20E-01	2.47E-01	-0.61
TS-10'	2.13E+02	5.29E-02	2.67E+02	7.55E-02	6.42E-02	-1.19
TS-30'	6.00E+01	1.49E-02	1.20E+02	3.39E-02	2.44E-02	-1.61
TS-60'	6.67E+00	1.66E-03	3.33E+01	9.42E-03	5.54E-03	-2.26
AVG VC	4.03E+03		3.54E+03			
VC-0'	5.13E+03		3.27E+03			
VC-60'	2.93E+03		3.80E+03			

VIRUS= POLIO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.26E+04	3.57E-01	1.05E+04	6.80E-01	5.18E-01	-0.29
TS-1'	1.05E+04	2.97E-01	1.23E+04	7.96E-01	5.47E-01	-0.26
TS-3'	1.07E+04	3.03E-01	9.80E+03	6.34E-01	4.69E-01	-0.33
TS-10'	1.11E+04	3.14E-01	1.26E+04	8.16E-01	5.65E-01	-0.25
TS-30'	6.80E+03	1.93E-01	6.13E+03	3.97E-01	2.95E-01	-0.53
TS-60'	2.80E+03	7.93E-02	3.20E+03	2.07E-01	1.43E-01	-0.84
AVG VC	3.53E+04		1.55E+04			
VC-0'	3.53E+04		1.53E+04			
VC-60'	3.53E+04		1.56E+04			

VIRUS = ECHO 1

SAMPLE	PFU/ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	9.40E+03	1.29E-01	5.67E+03	7.20E-02	1.00E-01	-1.00
TS-1'	3.60E+04	4.93E-01	1.43E+04	1.82E-01	3.37E-01	-0.47
TS-3'	5.33E+04	7.30E-01	4.00E+04	5.08E-01	6.19E-01	-0.21
TS-10'	3.47E+04	4.75E-01	3.20E+04	4.07E-01	4.41E-01	-0.36
TS-30'	2.53E+02	3.47E-03	4.13E+02	5.25E-03	4.36E-03	-2.36
TS-60'	4.00E+01	5.48E-04	3.33E+01	4.23E-04	4.86E-04	-3.31
AVG VC	7.30E+04		7.87E+04			
VC-0'	6.80E+04		7.27E+04			
VC-60'	7.80E+04		8.47E+04			

Table A44

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 7.0 AND 5C
IN WORST CASE WATER

AVG I2 CONCENTRATION 0'= 16.5 mg/L AVG HUMIC-FULVIC CONC= 10.4 mg/L
60'= 10.6 mg/L AVG BENTONITE CLAY= 5.5 NTU

VIRUS = HAV

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.00E+01	2.06E-03	1.27E+02	4.23E-02	2.22E-02	-1.65
TS-1'	< 5.00E+00	< 1.03E-03	< 5.00E+00	< 1.67E-03	< 1.35E-03	> -2.87
TS-3'						
TS-10'						
TS-30'						
TS-60'						
AVG VC	4.87E+03		3.00E+03			
VC-0'	4.00E+03		3.00E+03			
VC-60'	5.73E+03		3.00E+03			

VIRUS= POLIO 1

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	2.87E+04	6.06E-01	1.06E+04	2.69E-01	4.38E-01	-0.36
TS-1'	1.42E+04	3.00E-01	9.80E+03	2.49E-01	2.74E-01	-0.56
TS-3'	9.00E+03	1.90E-01	1.01E+04	2.57E-01	2.23E-01	-0.65
TS-10'	4.87E+03	1.03E-01	6.13E+03	1.56E-01	1.29E-01	-0.89
TS-30'	7.20E+02	1.52E-02	9.05E+02	2.30E-02	1.91E-02	-1.72
TS-60'	2.50E+01	5.28E-04	6.00E+01	1.52E-03	1.03E-03	-2.99
AVG VC	4.74E+04		3.94E+04			
VC-0'	5.27E+04		3.47E+04			
VC-60'	4.20E+04		4.40E+04			

VIRUS = ECHO 1

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	4.60E+03	6.45E-02	2.00E+03	3.37E-02	4.91E-02	-1.31
TS-1'	2.73E+04	3.83E-01	1.47E+04	2.48E-01	3.15E-01	-0.50
TS-3'	1.80E+04	2.52E-01	3.00E+04	5.06E-01	3.79E-01	-0.42
TS-10'	7.00E+01	9.82E-04	3.55E+02	5.99E-03	3.48E-03	-2.46
TS-30'	< 5.00E+00	< 7.01E-05	< 5.00E+00	< 8.43E-05	7.72E-05	> -4.11
TS-60'						
AVG VC	7.13E+04		5.93E+04			
VC 0'	6.93E+04		5.33E+04			
VC 60'	7.33E+04		6.53E+04			

Table A45

INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY 2 TABLETS/QUART IODINE AT pH 9.5 AND 5C IN WORST CASE WATER

AVG I2 CONCENTRATION 0'= 16.6 mg/L AVG HUMIC-FULVIC CONC=9.8 mg/L
30'= 2.2 mg/L AVG BENTONITE CLAY= 4.8 NTU

VIRUS = HAV

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	< 2.00E+00	< 1.83E-04	2.00E+00	1.59E-04	1.71E-04	> -3.77
TS-1'	< 2.00E+00	< 1.83E-04	< 2.00E+00	< 1.59E-04	< 1.71E-04	> -3.77
TS-3'	4.00E+00	3.66E-04	2.00E+00	1.59E-04	2.63E-04	-3.58
TS-10'	4.00E+00	3.66E-04	< 2.00E+00	< 1.59E-04	< 2.63E-04	> -3.58
TS-30'	2.00E+00	1.83E-04	4.00E+00	3.17E-04	2.50E-04	-3.60
AVG VC	1.09E+04		1.26E+04			
VC-0'	1.49E+04		1.21E+04			
VC-30'	6.93E+03		1.31E+04			

VIRUS= POLIO 1

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	1.73E+02	1.43E-02	1.63E+03	1.55E-01	8.45E-02	-1.07
TS-1'	1.47E+02	1.21E-02	1.33E+02	1.26E-02	1.24E-02	-1.91
TS-3'	3.47E+02	2.87E-02	4.27E+02	4.05E-02	3.46E-02	-1.46
TS-10'	9.33E+01	7.71E-03	6.67E+01	6.33E-03	7.02E-03	-2.15
TS-30'	2.67E+01	2.21E-03	9.33E+01	8.86E-03	5.53E-03	-2.26
AVG VC	1.21E+04		1.05E+04			
VC-0'	1.03E+04		1.28E+04			
VC-30'	1.39E+04		8.27E+03			

VIRUS = ECHO 1

SAMPLE	PFU /ML	Nt/No	PFU /ML	Nt/No	AVG Nt/No	LOG AVG Nt/No
TS-20"	8.53E+04	9.77E-01	7.07E+04	1.29E+00	1.14E+00	0.06
TS-1'	1.33E+01	1.52E-04	4.00E+01	7.32E-04	4.42E-04	-3.35
TS-3'	2.67E+01	3.06E-04	< 1.33E+01	< 2.44E-04	< 2.75E-04	> -3.56
TS-10'	< 1.33E+01	< 1.53E-04	< 1.33E+01	< 2.44E-04	< 1.98E-04	> -3.70
TS-30'						
AVG VC	8.73E+04		5.47E+04			
VC-0'	8.13E+04		4.93E+04			
VC-30'	9.33E+04		6.00E+04			

APPENDIX B.

Statistical Analysis Data Tables

Table I. Ranked data from halogen demand free and worst case water experiments[a].

Virus	Temp[b]	Conc[c]	pH	Water Quality[d]	Rank[e]	Detection Limit[f]
Echo	25	7	7.0	2	10	0.33
HAV	25	7	7.0	2	10	0.33
HAV	25	1	7.0	1	10	0.33
HAV	25	7	4.5	2	10	0.33
Polio	25	7	7.0	2	10	0.33
Polio	25	3	7.0	2	10	0.33
Polio	25	7	9.5	2	10	0.33
Polio	25	7	4.5	2	10	0.33
Polio	25	5	4.5	1	10	0.33
Echo	25	7	4.5	2	10	0.33
Polio	25	5	7.0	1	10	0.33
HAV	5	3	7.0	2	10	0.33
HAV	25	3	7.0	2	10	0.33
HAV	25	7	9.5	2	10	0.33
Echo	25	3	4.5	2	10	0.33
Echo	25	5	7.0	1	10	0.33
HAV	25	3	9.5	2	10	0.33
Echo	25	5	4.5	1	10	0.33
HAV	25	5	7.0	1	10	0.33
Echo	25	7	9.5	2	32.5	1.00
Polio	5	7	7.0	2	32.5	1.00
Polio	25	1	7.0	1	32.5	1.00
Echo	5	7	4.5	2	32.5	1.00
Echo	5	5	4.5	1	32.5	1.00
Echo	25	3	7.0	2	32.5	1.00
HAV	5	3	9.5	2	32.5	1.00
HAV	25	3	4.5	2	32.5	1.00
Echo	5	7	7.0	2	32.5	1.00
Polio	5	5	4.5	1	32.5	1.00
HAV	25	5	4.5	1	32.5	1.00
HAV	5	7	9.5	2	32.5	1.00
Echo	5	5	7.0	1	32.5	1.00
HAV	5	7	4.5	2	32.5	1.00
Polio	25	3	4.5	2	32.5	1.00
Polio	5	3	7.0	2	32.5	1.00
HAV	5	1	7.0	1	32.5	1.00
Polio	5	7	4.5	2	32.5	1.00
Polio	25	1	4.5	1	32.5	1.00
Echo	25	1	4.5	1	32.5	1.00
HAV	25	5	9.5	1	32.5	1.00
Polio	25	3	9.5	2	32.5	1.00
Echo	25	1	7.0	1	32.5	1.00
HAV	5	7	7.0	2	32.5	1.00
Echo	5	3	7.0	2	32.5	1.00
Echo	5	3	4.5	2	32.5	1.00

continued on next page.

(Table I. continued) Ranked data from halogen demand free and worst case water experiments[a].

Virus	Temp[b]	conc[c]	pH	Water Quality[d]	Rank[e]	Detection Limit[f]
HAV	5	5	7.0	1	51	3.00
Polio	5	3	4.5	2	51	3.00
Polio	5	5	7.0	1	51	3.00
Polio	5	1	7.0	1	51	3.00
HAV	5	5	4.5	1	51	3.00
Polio	5	7	9.5	2	51	3.00
HAV	5	3	4.5	2	51	3.00
HAV	25	1	9.5	1	51	3.00
HAV	5	5	9.5	1	51	3.00
Polio	25	5	9.5	1	51	3.00
HAV	25	1	4.5	1	51	3.00
Echo	5	1	7.0	1	62	10
Echo	5	7	9.5	2	62	10
Polio	5	3	9.5	2	62	10
Polio	5	1	4.5	1	62	10
HAV	5	1	4.5	1	62	10
Echo	25	3	9.5	2	62	10
Polio	5	5	9.5	1	62	10
Echo	5	1	4.5	1	62	10
HAV	5	1	9.5	1	62	10
Polio	25	1	9.5	1	62	10
Echo	25	5	9.5	1	62	10
Echo	25	1	9.5	1	69	30
Echo	5	5	9.5	1	69	30
Echo	5	3	9.5	2	69	30
Polio	5	1	9.5	1	71	60
Echo	5	1	9.5	1	72	>60

a. Data for each virus at a specfic temperature, free chlorine concentration, pH and water quality were ranked by their detection limit point.
b. Temperature in degrees centigrade.
c. Initial free chlorine concentration in mg/L.
d. Water quality: halogen demand free water = 1; worst case water = 2.
e. Tied observations were assigned the average of the ranks that would be assigned if there were no ties.
f. Detection limit in minutes.

TABLE II. Kruskall-Wallis One-Way Analysis of Variance for Free Chlorine Disinfection Experiments: Data Summary for Test Variables

Variable	Test Statistic (H)	Probability (P)
Temperature	1016	<0.001
pH	15.7	<0.001
Chlorine Concentration	14.8	0.002
Water Quality	9.04	0.003
Virus Type	1.98	0.372

*Number of observations or samples (n) = 72.

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